KSBi-BIML 2024

Bioinformatics & Machine Learning(BIML)
Workshop for Life and Medical Scientists

생명정보학 & 머신러닝 워크샵 (온라인)

(Single-cell) 3D Epigenome Data Analysis

정인경 _ KAIST

KSBI KOREAN SOCIETY FOR BIOINFORMATICS | 한국생명정보학회

본 강의 자료는 한국생명정보학회가 주관하는 BIML 2024 워크샵 온라인 수업을 목적으로 제작된 것으로 해당 목적 이외의 다른 용도로 사용할 수 없음을 분명하게 알립니다.

이를 다른 사람과 공유하거나 복제, 배포, 전송할 수 없으며 만약 이러한 사항을 위반할 경우 발생하는 **모든 법적 책임은 전적으로 불법 행위자 본인에게 있음을 경고**합니다.

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안녕하십니까?

한국생명정보학회가 개최하는 동계 교육 워크샵인 BIML-2024에 여러분을 초대합니다. 생명정보학 분야의 연구자들에게 최신 동향의 데이터 분석기술을 이론과 실습을 겸비해 전달하고자 도입한 전문 교육 프로그램인 BIML 워크샵은 2015년에 시작하여 올해로 벌써 10년 차를 맞이하게 되었습니다. BIML 워크샵은 국내 생명정보학 분야의 최초이자 최고 수준의 교육프로그램으로 크게 인공지능과 생명정보분석 두 개의 분야로 구성되어 있습니다. 올해 인공지능 분야에서는 최근 생명정보 분석에서도 응용이 확대되고 있는 다양한 인공지능 기반 자료모델링 기법들에 대한 현장 강의가 진행될 예정이며, 관련하여 심층학습을 이용한 단백질구조예측, 유전체분석, 신약개발에 대한 이론과 실습 강의가 함께 제공될 예정입니다. 또한 단일세포오믹스, 공간오믹스, 메타오믹스, 그리고 롱리드염기서열 자료 분석에 대한 현장 강의는 많은 연구자의 연구 수월성 확보에 큰 도움을 줄 것으로 기대하고 있습니다.

올해 BIML의 가장 큰 변화는 최근 연구 수요가 급증하고 있는 의료정보자료 분석에 대한 현장 강의를 추가하였다는 것입니다. 특히 의료정보자료 분석을 많이 수행하시는 의과학자 및 의료정보 연구자들께서 본 강좌를 통해 많은 도움을 받으실 수 있기를 기대하고 있습니다. 또한 다양한 생명정보학 분야에 대한 온라인 강좌 프로그램도 점차 증가하고 있는 생명정보 분석기술의 다양화에 발맞추기 위해 작년과 비교해 5강좌 이상을 신규로 추가했습니다. 올해는 무료 강좌 5개를 포함하여 35개 이상의 온라인 강좌가 개설되어 제공되며, 연구 주제에 따른 연관된 강좌 추천 및 강연료 할인 프로그램도 제공되며, 온라인을 통한 Q&A 세션도 마련될 예정입니다. BIML-2024는 국내 주요 연구중심 대학의 전임 교원이자 각 분야 최고 전문가들의 강의로 구성되었기에 해당 분야의 기초부터 최신 연구 동향까지 포함하는 수준 높은 내용의 강의가 될 것이라 확신합니다.

BIML-2024을 준비하기까지 너무나 많은 수고를 해주신 운영위원회의 정성원, 우현구, 백대현, 김태민, 김준일, 김상우, 장혜식, 박종은 교수님과 KOBIC 이병욱 박사님께 커다란 감사를 드립니다. 마지막으로 부족한 시간에도 불구하고 강의 부탁을 흔쾌히 허락하시고 훌륭한 현장 강의와 온라인 강의를 준비하시는데 노고를 아끼지 않으신 모든 강사분들께 깊은 감사를 드립니다.

2024년 2월

한국생명정보학회장 이 인 석

강의개요

(Single-cell) 3D Epigenome Data Analysis

염색질 3차구조란 핵 안에 3차원으로 배열된 게놈의 구조를 의미한다. 최근 연구 결과에 따르면 염색질 3차 구조는 무작위적 배열보다는 TAD (Topologically Associating Domain) 또는 Loop domain을 기본 단위로 여러 계층으로 구성되어 있으며, 이러한 구조적 제약에 의해 DNA 서열상 멀리 떨어진 인핸서, 프로모터 등 여러 전사 조절 인자들은 3차원 공간상에 인접할 수 있게 되어 전사 조절의 핵심 원리로 제시되고 있다. 이러한 염색질 3차구조는 게놈의 후성유전적 변화와 밀접한 연관이 있기에 최근 염색질 3차구조와 게놈의 후성유전적 변화를 통합 분석하려는 '3D epigenome' 연구가 급격하게 발전하고 있다.

본 강의에서는 염색질 3차구조를 중심으로 관련 이론, 실험 방법, 그리고 기본 데이터 분석을 실습과 함께 숙지하고자 한다. 간략하게 후성유전학 대한 강의 후, 염색질 3차구조에 대한 전반적인 소개, 그리고 최신 급격하게 발전중에 있는 염색질 3차구조 중심의 단일세포 multi-omics관련 연구를 소개하고 이들 데이터 분석 workflow에 대해 배우고자 한다. 또한 본 연구팀이 개발한 3DIV 웹기반 염색질 3차구조 데이터 분석 실습을 통해 Hi-C 데이터 분석을 배워보고자 한다.

강의는 다음의 내용을 포함한다:

- 후성유전학/염색질 3차구조 개요
- 염색질 3차구조 중심의 단일세포 multi-omics 개요
- 염색질 3차구조 데이터 분석 방법
- 3DIV 기반 Hi-C 데이터 분석 실습

* 교육생준비물: 노트북 (메모리 8GB 이상, 디스크 여유공간 30GB 이상)

* 강의: 정인경 교수 (한국과학기술원 생명과학과)

Curriculum Vitae

Speaker Name: Inkyung Jung, Ph.D.

▶ **Personal Info**

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Affiliation	KAIST

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Research Interest

Epigenetic gene regulation, 3D chromatin structure, single-cell multi-omics

Educational Experience

2006-2011	Ph.D. KAIST / Bio and Brain Engineering
2002-2006	B.S. KAIST / Biosystems

Professional Experience

2016-present	Assistant Professor, Associate Professor, Department of Biological Sciences, KAIST
2012-2016	Postdoctoral fellow, Ludwig Institute for Cancer Research
2011-2012	Postdoctoral fellow, KAIST

Selected Publications (5 maximum)

1. Kim K*, Kim M*, Lee AJ*, Song SH*, Kang JK, Eom J, Kang GH, Bae JM, Min S, Kim Y, Lim Y, Kim HS, Kim YJ, Kim TY#, Jung I# (**2023**) Spatial and clonality-resolved 3D cancer genome alterations reveal enhancer-hijacking as a potential prognostic marker for colorectal cancer. ***Cell Rep***. Jul 25;42(7):112778
2. Lee AJ*, Kim C*, Park S, Jun K, Eom J, Lee S-J, Chung SJ, Rissman RA, Chung J, Masliah E#, Jung I# (**2023**) Characterization of altered molecular mechanisms in Parkinson's disease through cell type-resolved multi-omics analyses. ***Sci Adv***. Apr 14;9(15):eabo2467
3. Joo J*, Cho S*, Hong S, Min S, Kim K, Kumar R, Choi J, Shin Y#, Jung I# (**2023**) Probabilistic establishment of speckle-associated inter-chromosomal interactions, ***Nucleic Acids Res***. Apr 4;gkad211
4. Kim K*, Jang I*, Kim M*, Choi J, Kim MS, Lee B#, Jung I# (**2021**) 3DIV Update for 2021: a comprehensive resource of 3D genome and 3D cancer genome. ***Nucleic Acids Res***. Jan 8;49(D1):D38-D46
5. Jung I*#, Schmitt A*, Diao Y*, Lee AJ, Liu T, Yang D, Tan C, Eom J, Chan M, Chee S, Chiang Z, Kim C, Masliah E, Barr CL, Li B, Kuan S, Kim D, Ren B#. (**2019**) A Compendium of Promoter-Centered Long-Range Chromatin Interactions in the Human Genome. ***Nat Genet***. Oct;51(10):1442-1449

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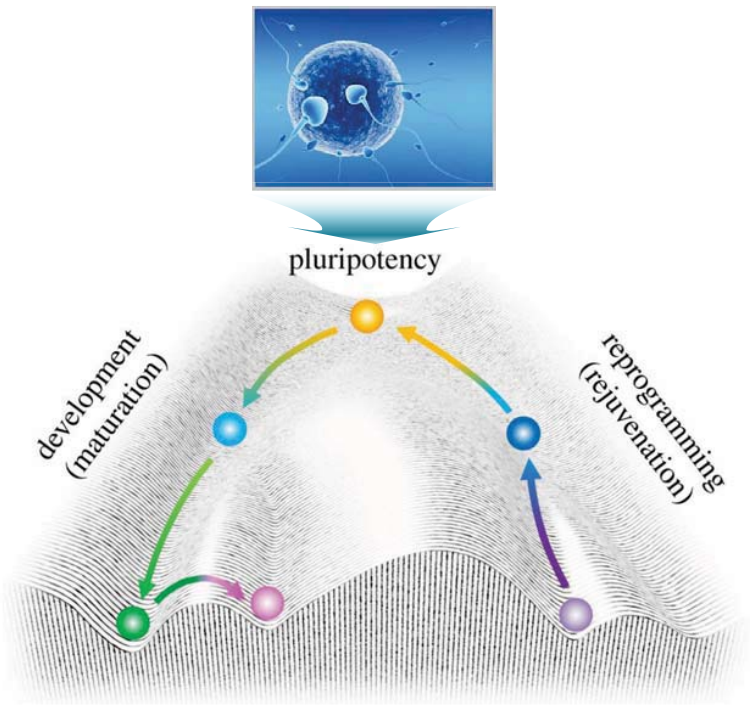
KSBi-BIML 2024

(Single-cell) 3D Epigenome Data Analysis

정인경(KAIST)

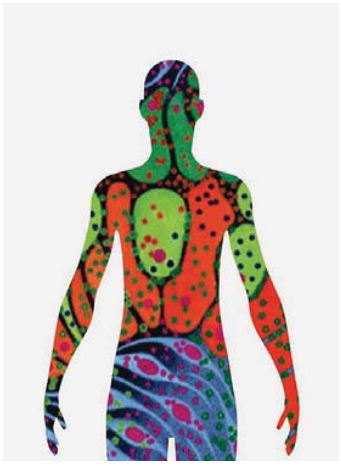
Contents

Epigenetic gene regulation determines cell fate

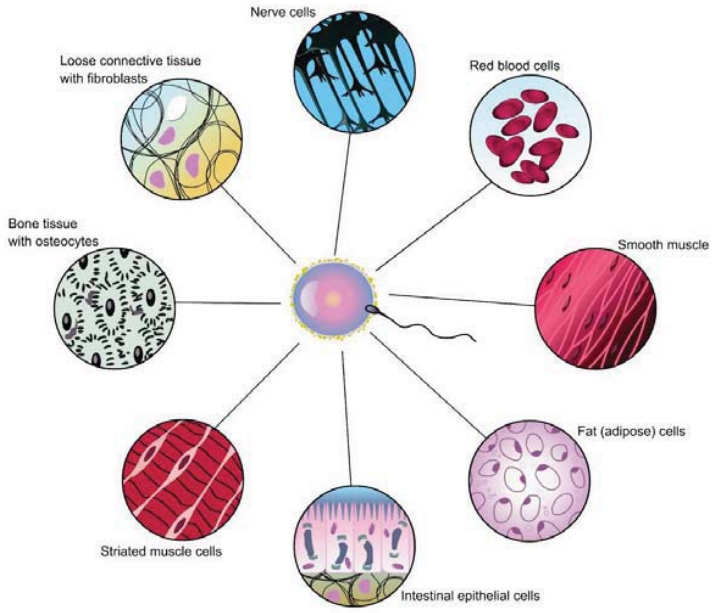


Waddington's epigenetic landscape (Evolution, 1956)

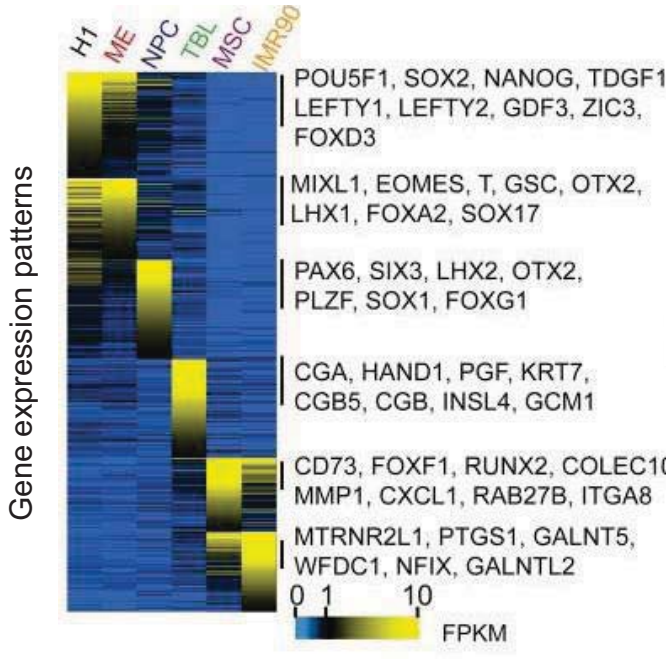
One genome, but many different functions



30 trillion cells



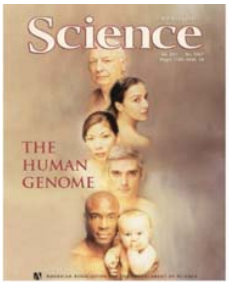
Cell-type specific transcriptome determines cell-type specific function

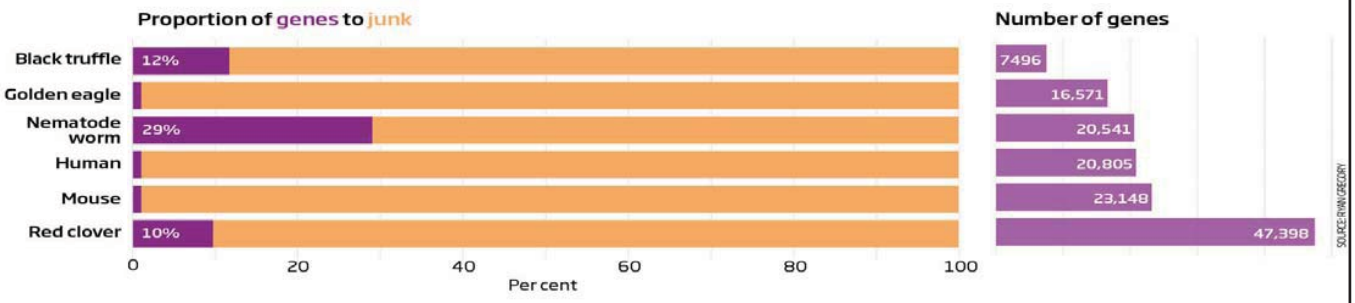


Cell type specific gene expression patterns can characterize cellular identity and define cell type specific biological functions

Human Genome project (1990-2003)

Before sequencing human genome scientists estimated the number of genes in human genome as 1,000,000





Protein-coding genes occupy a small fraction of the human genome
– no more than about 2-3% -

Systematic characterization of non-coding sequences

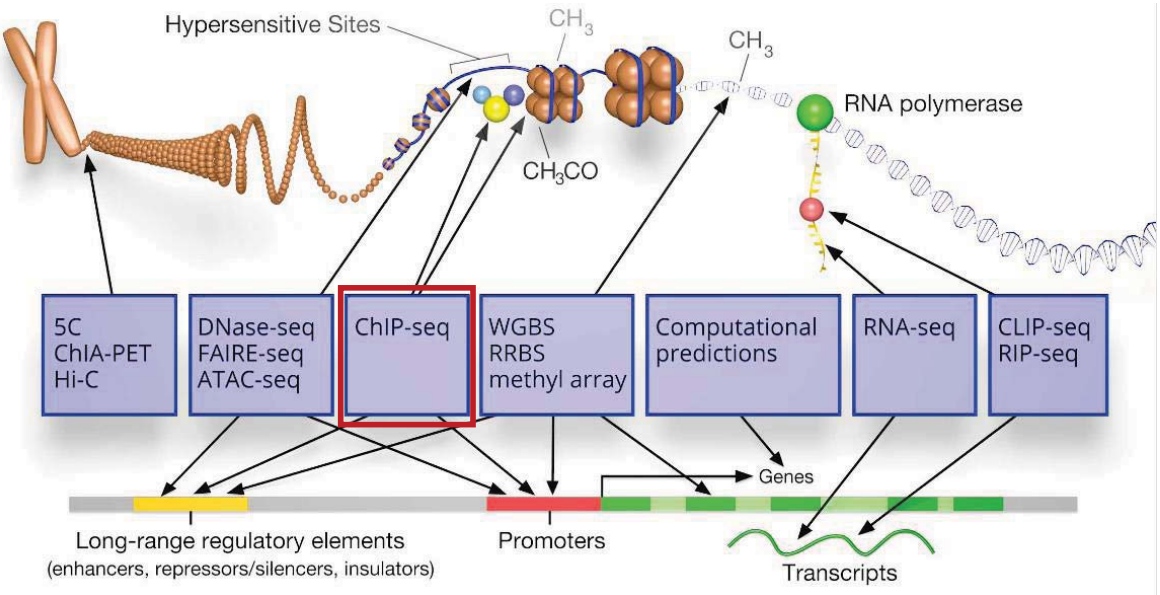
ENCODE consortium (2012)

Roadmap Epigenome consortium (2015)

ENCODE / Roadmap Epigenomics

- **Encyclopedia of DNA Elements (ENCODE) : a public research project launched in 2003 (mostly cell lines)**
- *"aims to identify all functional elements in the human genome sequence."*

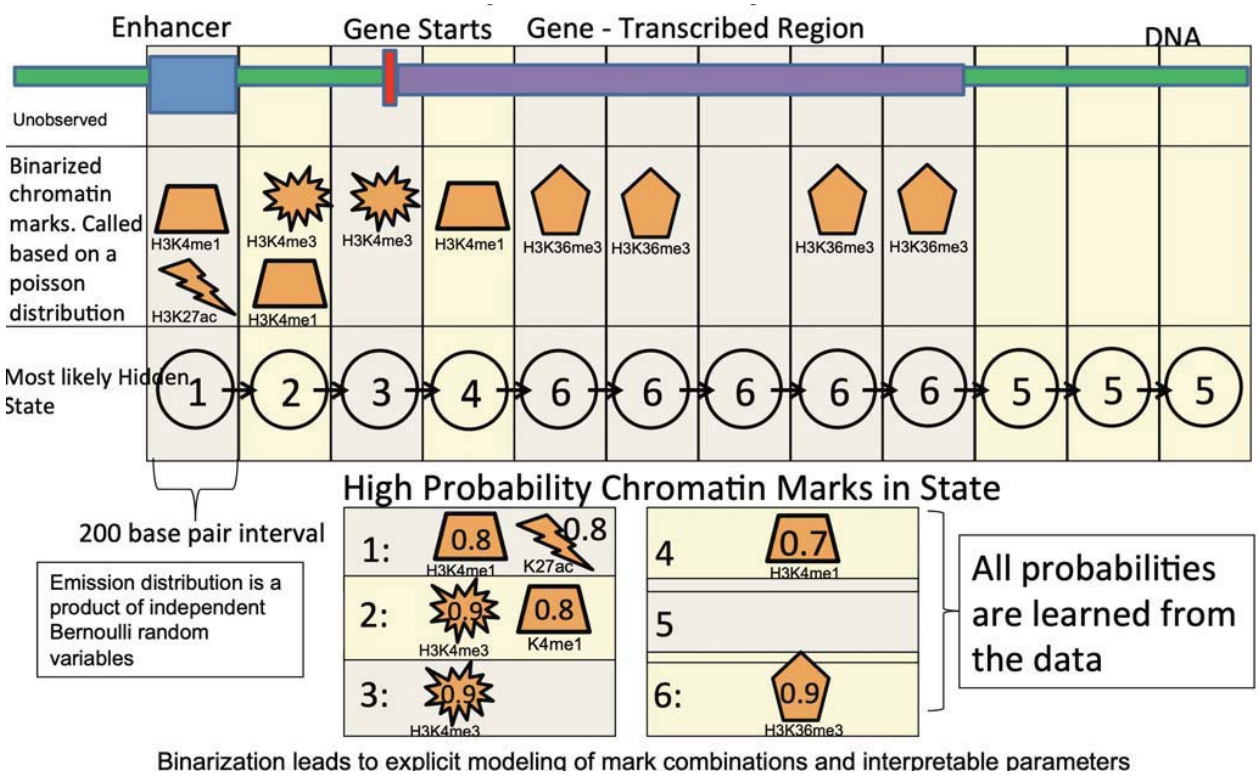
- **Roadmap Epigenomics: Launched in 2008 (mostly primary human tissues)**
- *"aims to produce a public resource of epigenomic maps for stem cells and primary ex vivo tissues selected to represent the normal counterparts of tissues and organ systems frequently involved in human disease."*



WashU epigenome browser (https://epigenomegateway.wustl.edu/)

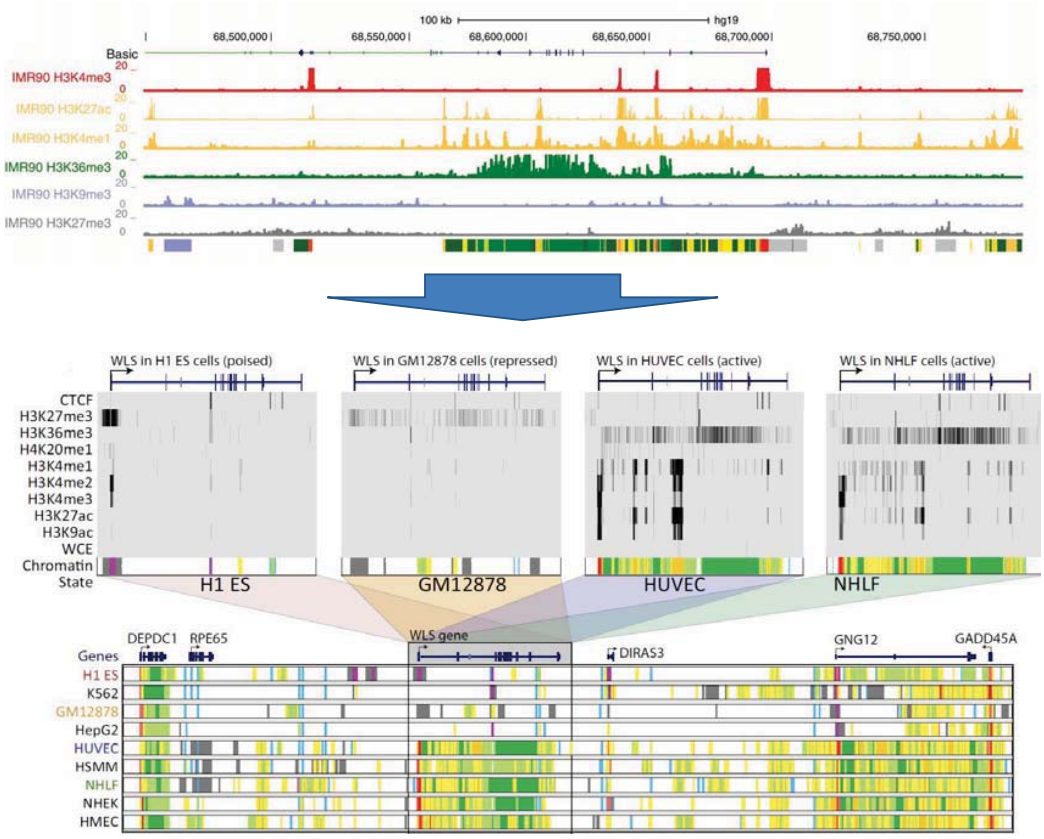
Annotation track for functional elements

A ChromHMM model to systematically annotate various chromatin state



Roadmap Epigenomics (2015)

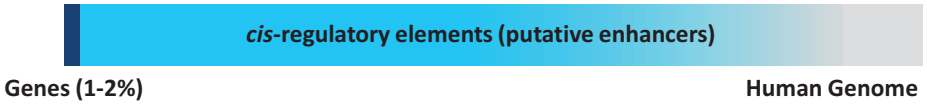
A ChromHMM model to systematically annotate various chromatin state



Nature (2011)

What have we learned from ENCODE/Roadmap Epigenomics?

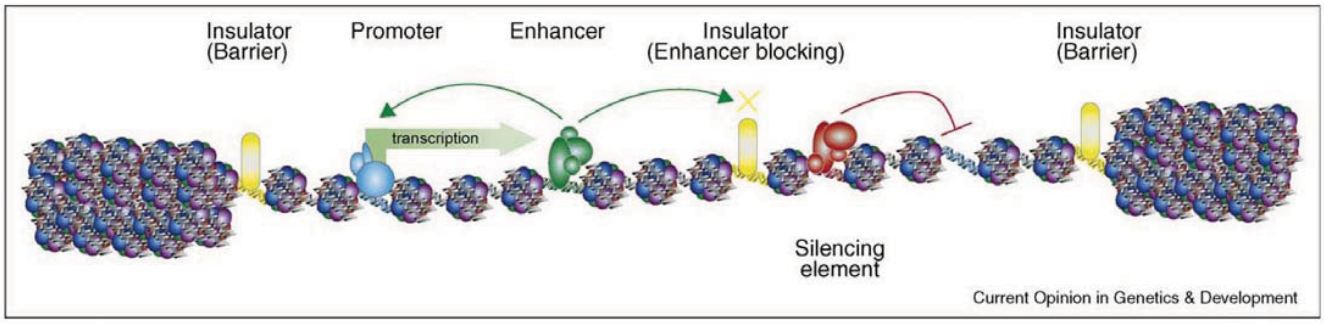
1. 80.4% of the human genome participates in at least one biochemical chromatin associated events

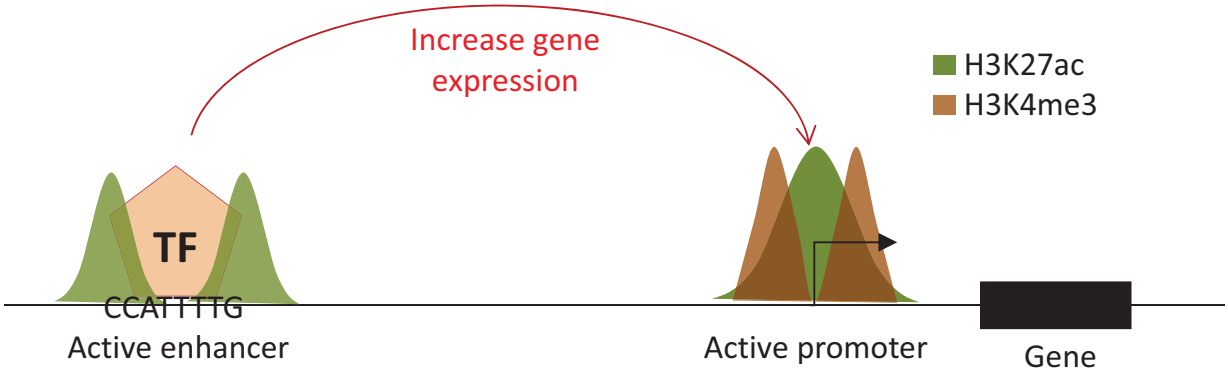


2. Many important genetic variants are found at cis-regulatory elements

3. Enhancer elements are the major player in cell-type specific gene regulation

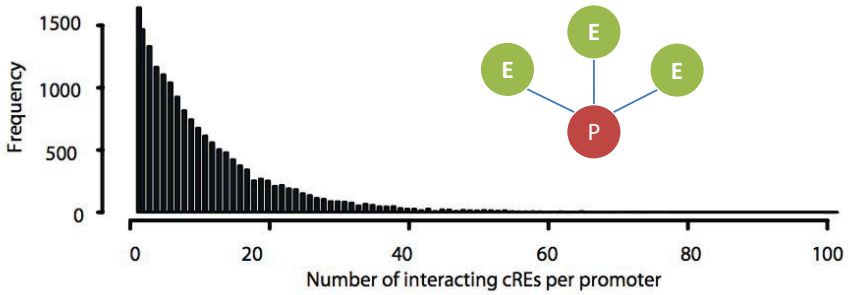
"Enhancer" is a major player in epigenetic gene regulation

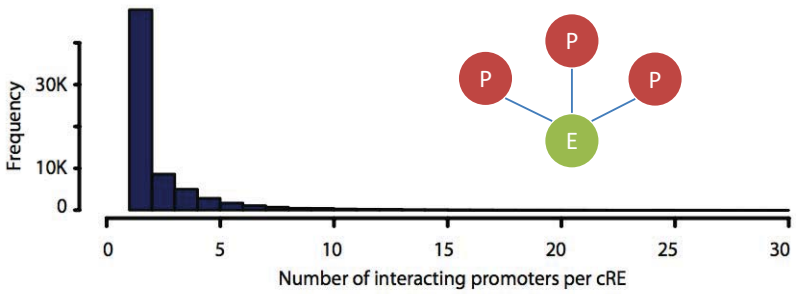




A epigenetic switch of gene expression

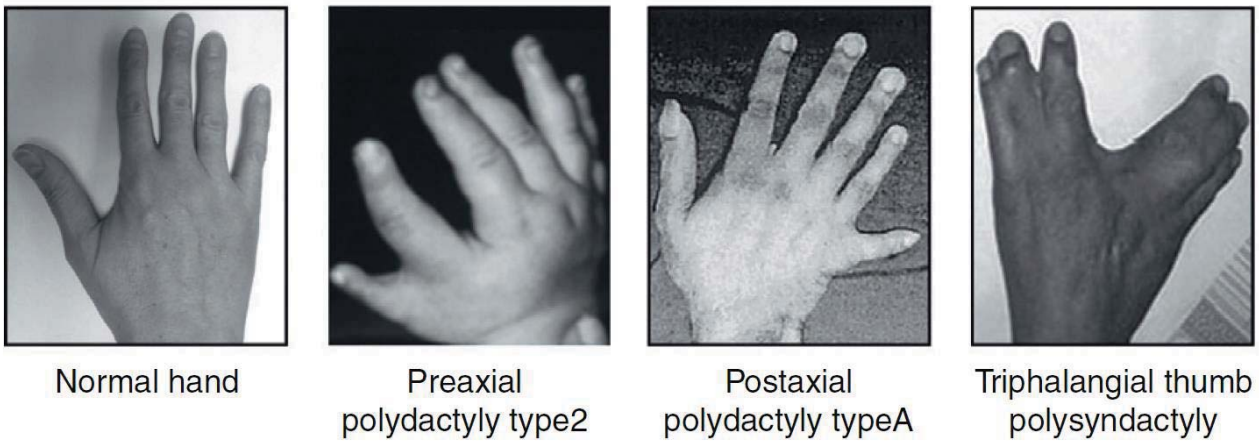
One promoter can be controlled by multiple enhancers



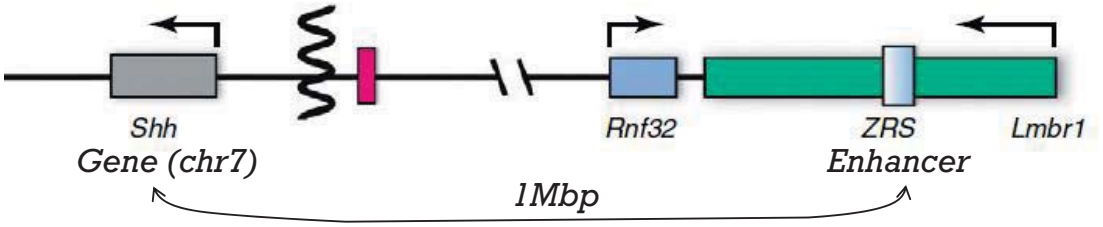


Enhancers can control distal target gene expression

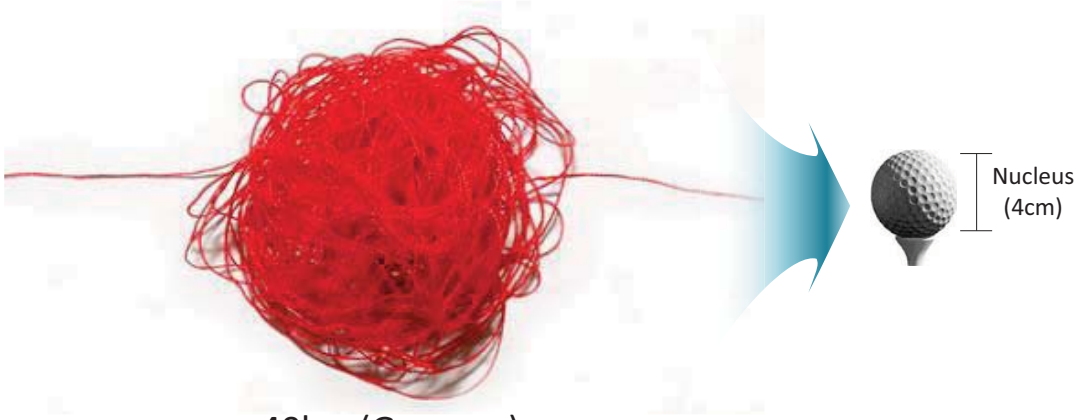
Polydactyly syndrome



Normal hand | Preaxial polydactyly type2 | Postaxial polydactyly typeA | Triphalangial thumb polysyndactyly

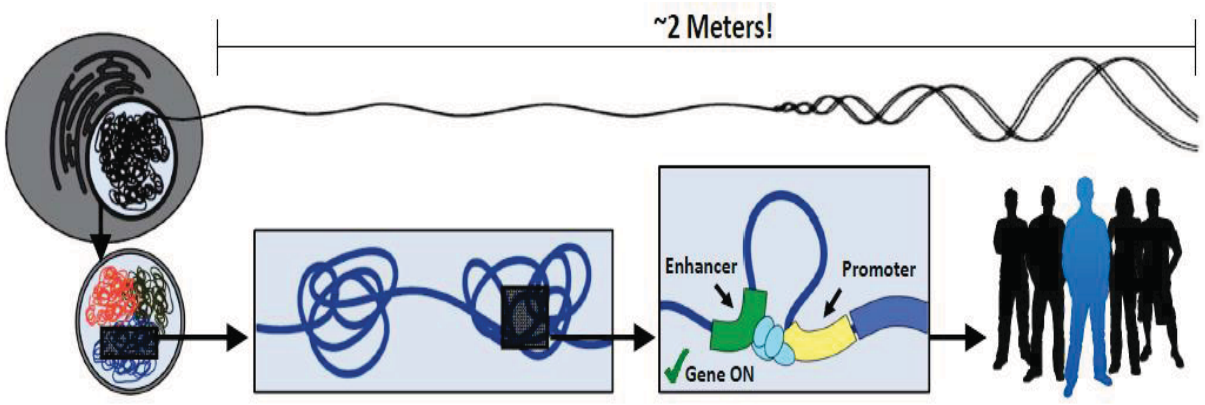


How does enhancer control distal gene expression?



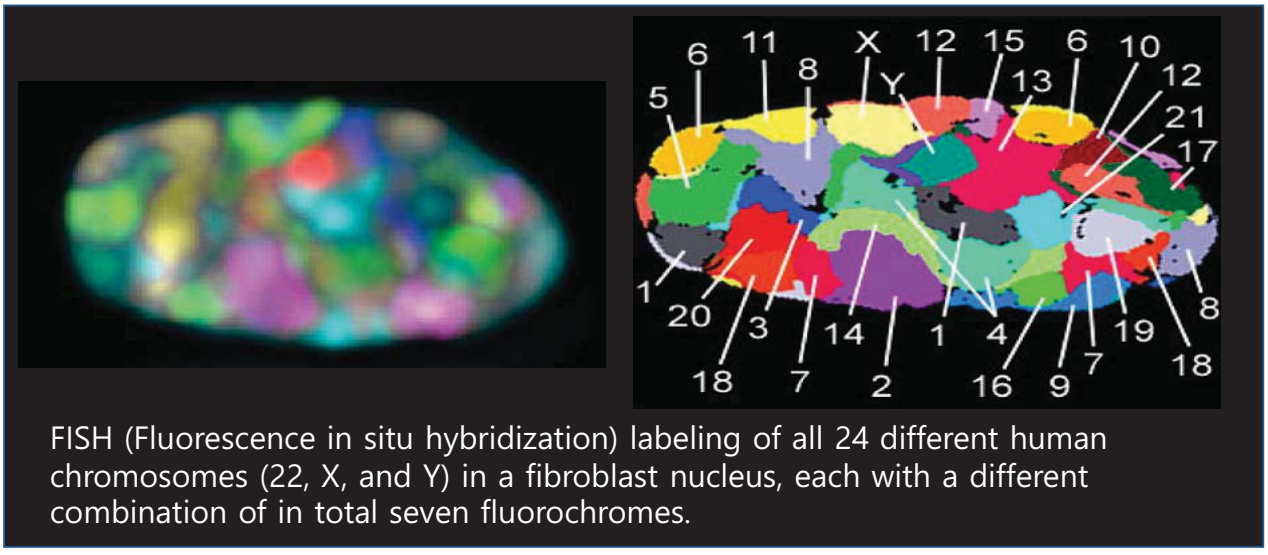
40km (Genome)

Chromatin is not randomly folded into the nucleus



- Human DNA is well packaged
  - Length = near 2m
  - Average human cell nucleus : 6 micron → 1/300,000 compaction
- Chromatin is not randomly folded into the nucleus

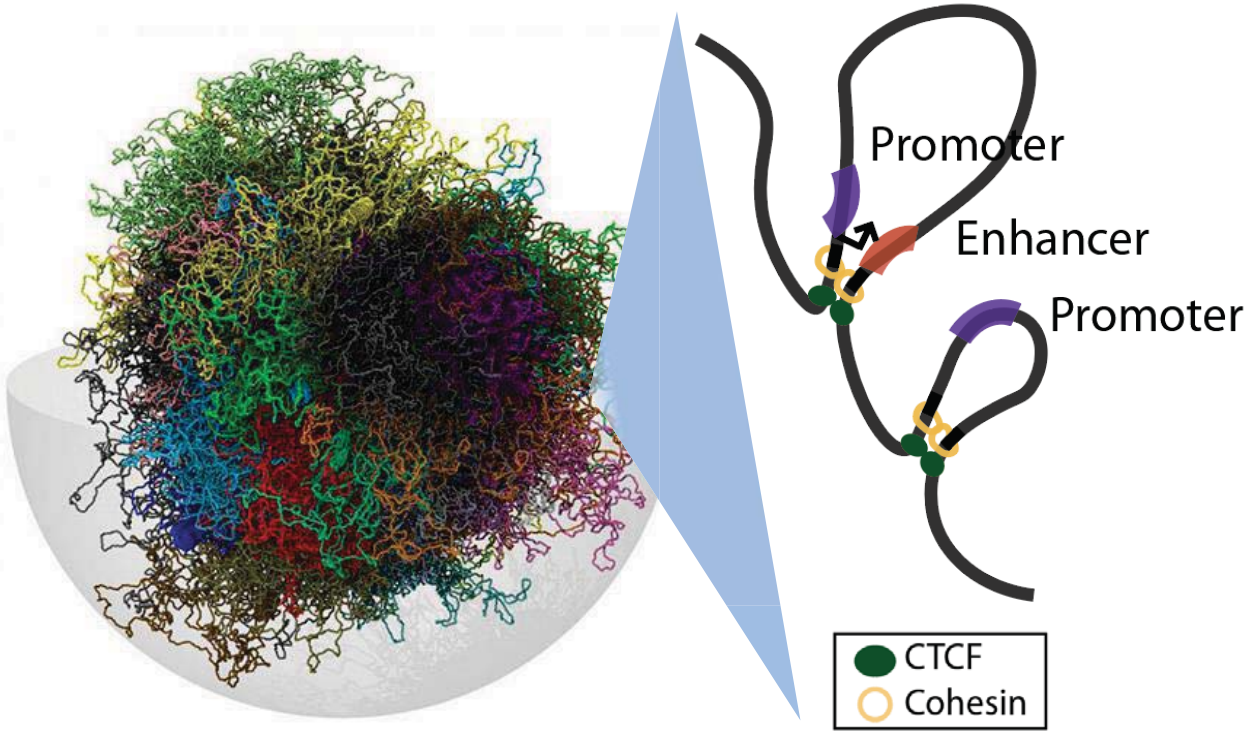
A theory of chromosome territory



FISH (Fluorescence in situ hybridization) labeling of all 24 different human chromosomes (22, X, and Y) in a fibroblast nucleus, each with a different combination of in total seven fluorochromes.

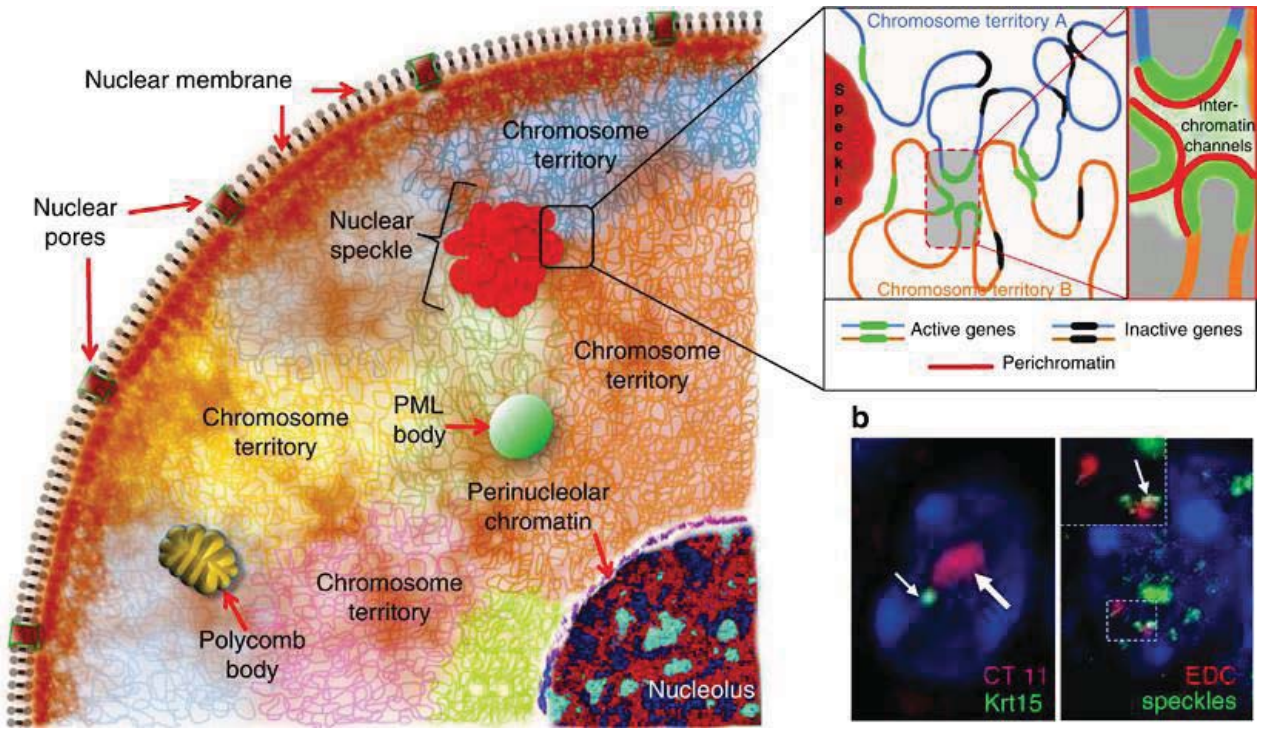
Bolzer et al., (2005)

3D genome enables enhancers control distal gene expression



3D genome: A spatial arrangement of the genome where distant DNA fragments can be juxtaposed in nuclear space

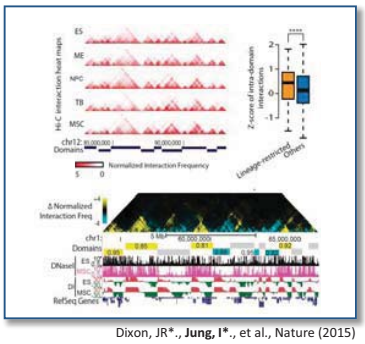
Genome organization in 3D nuclear space



Botchkarev et al., 2012

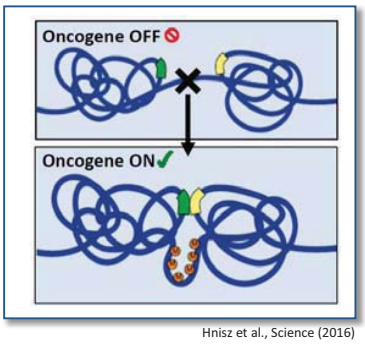
Genome functions are tightly coupled with 3D chromatin structure

Cellular differentiation



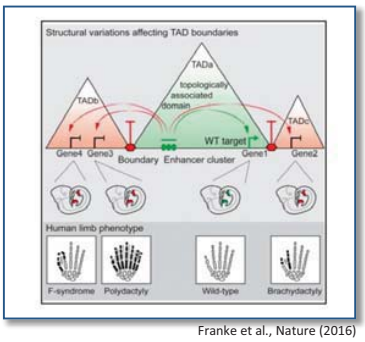
Dixon, JR*., **Jung, I***., et al., Nature (2015)

Oncogene activation



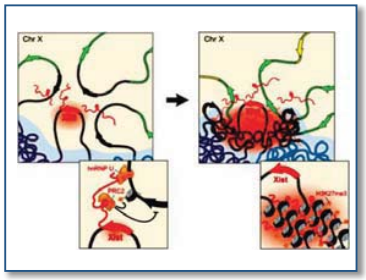
Hnisz et al., Science (2016)

Congenital disorder



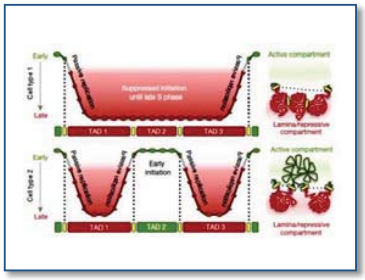
Franke et al., Nature (2016)

X-chromosome inactivation



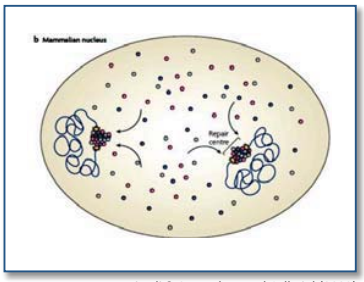
Engreitz et al., Science (2013)

DNA replication



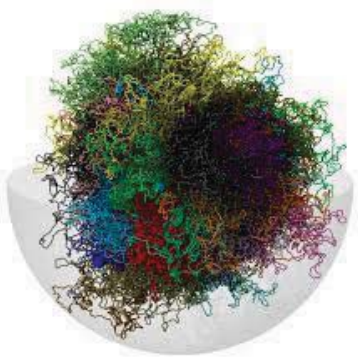
Pope et al., Nature (2014)

DNA repair



Misteli & Soutoglou, Mol Cell Biol (2009)

Methods to detect 3D genome organization



Imaging based methods:

1. Electron microscopy : labor intensive and not easily applicable to studies of specific loci
2. Light microscopy: Limited resolution (100~200 nm) to define chromosome conformation.
3. FISH (fluorescence in situ hybridization): Requires severe treatment that may affect chromosome organization

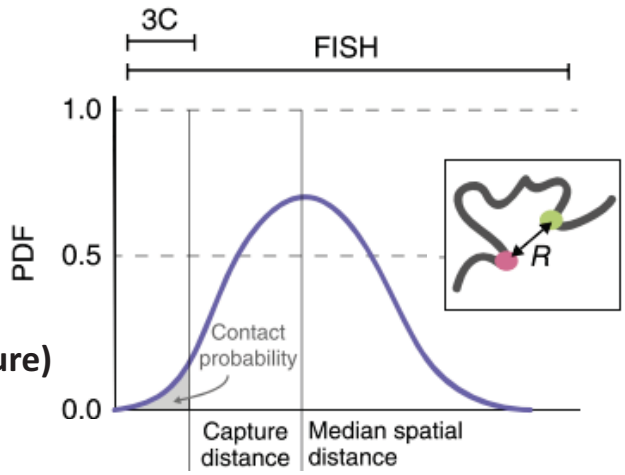
Require alternative strategies to detect chromatin interactions at high-resolution genome-wide

3C	5C	4C-seq	ChIA-PET	Hi-C	Capture HiC	HiChIP	GAM	LAD

Imaging vs sequencing methods

Imaging (FISH)

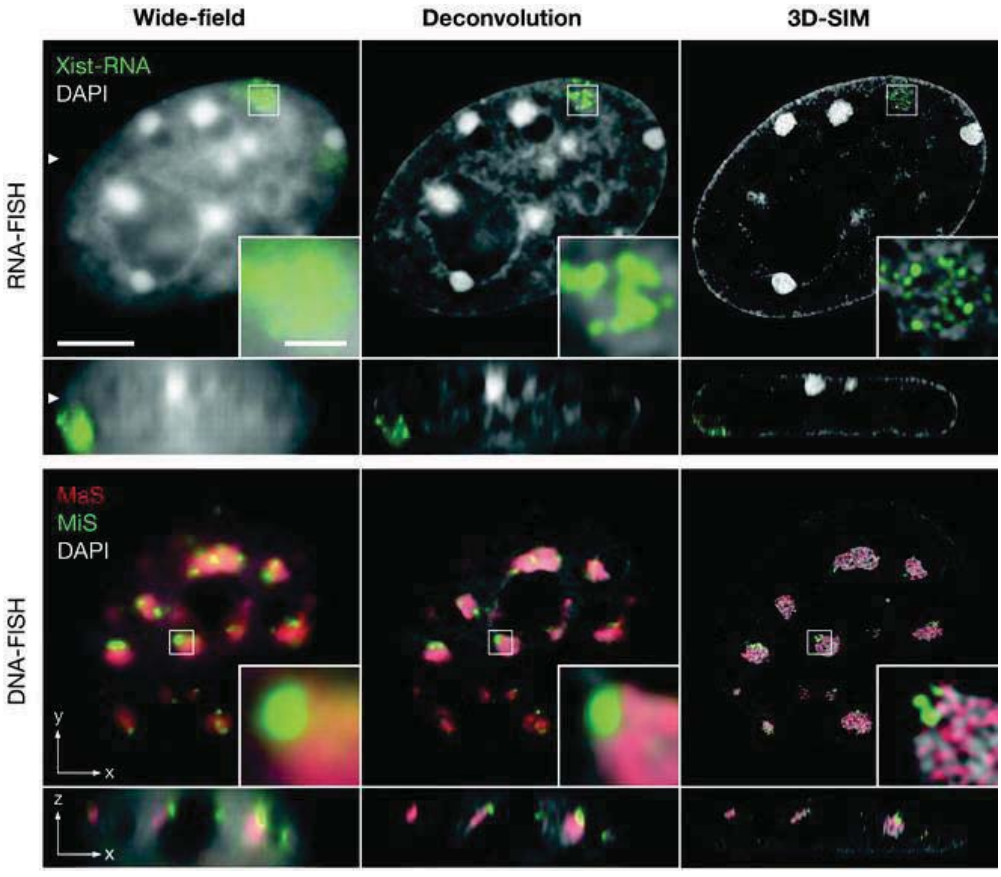
- In general: Single cell
- **Spatial distance**
  - Any distance outside probe “glare”



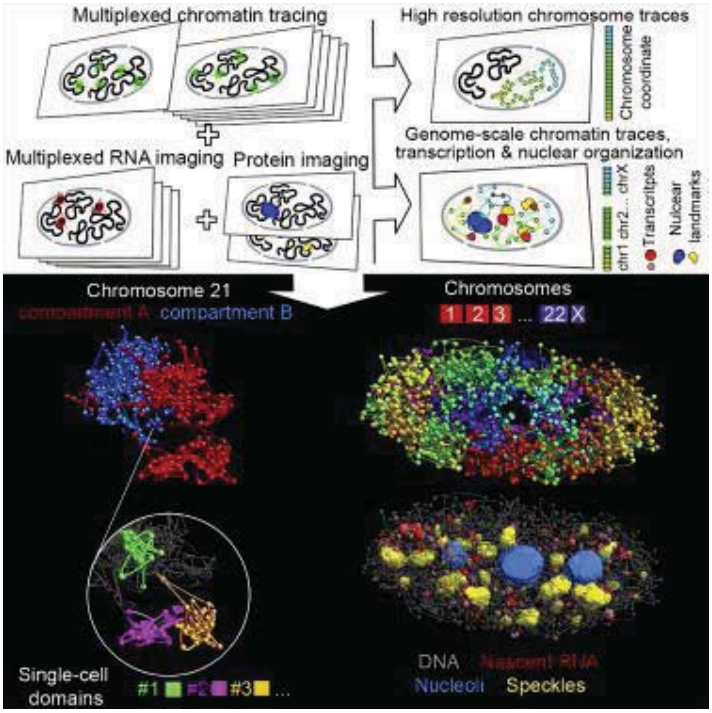
Omics (Chromosome Conformation Capture)

- In general: population
- **Contact frequency**
  - Capture radius dependent
  - Long distances in close proximity

Belmont, *Curr.Opin.Cell Biol*. 2014
Giorgietti, *Gen.Biol* 2016
Fudenberg and Imakaev, *Nat.Methods* 2017



https://link.springer.com/protocol/10.1007/978-1-62703-137-0_4

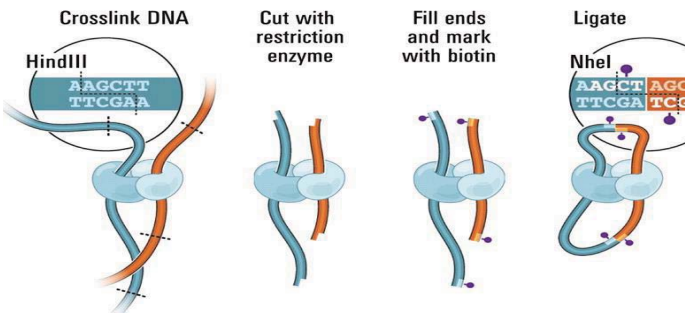


A multiplexed error-robust fluorescence *in situ* hybridization (MERFISH)

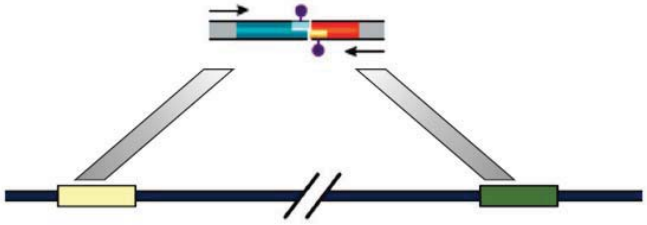
https://www.sciencedirect.com/science/article/pii/S0092867420309405

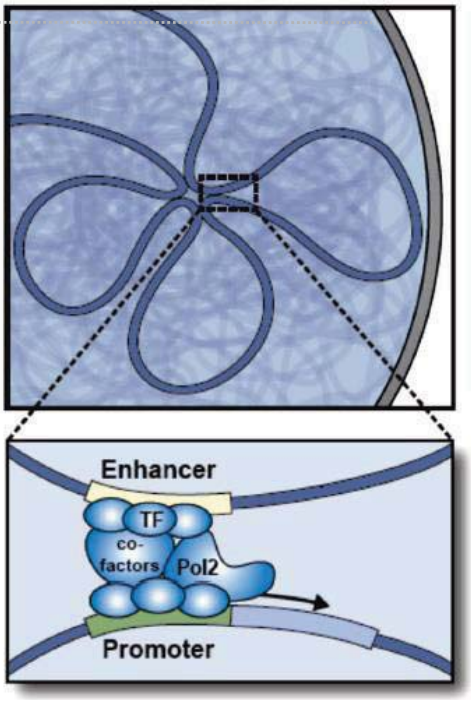
How can we investigate 3D genome organization?

Hi-C: **Hi**gh-throughput **c**hromosome conformation capture (3C)



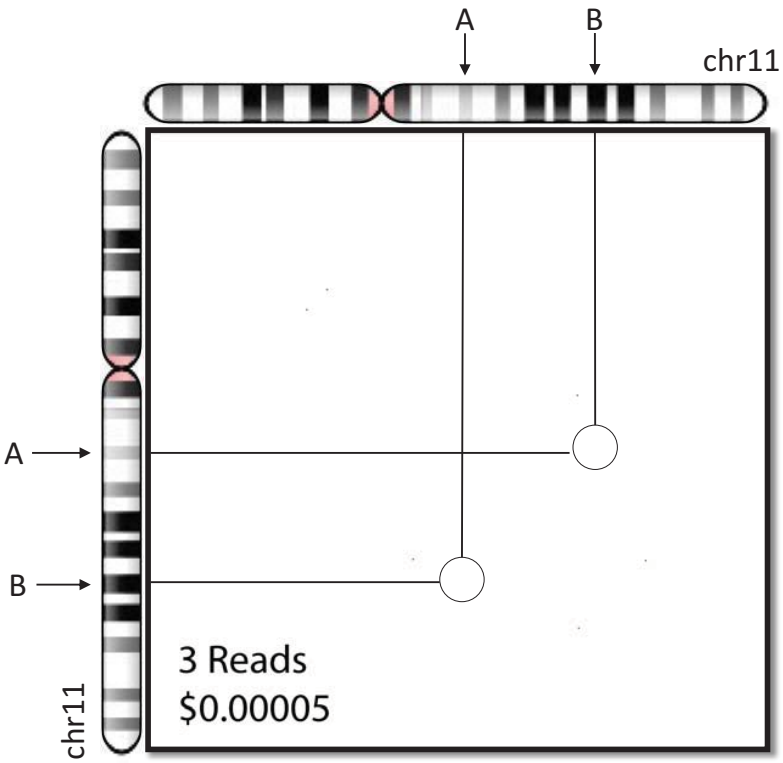
Cross-linking → Digestion → Re-lig





Lieberman et al., Science (2009)

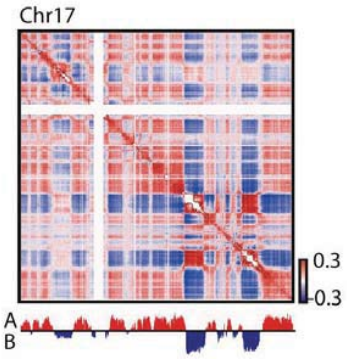
Hi-C contact map to visualize 3D genome



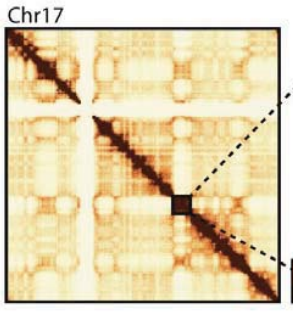
cis-chromosomal interactions (chr11)

Multi-layered 3D genome organization

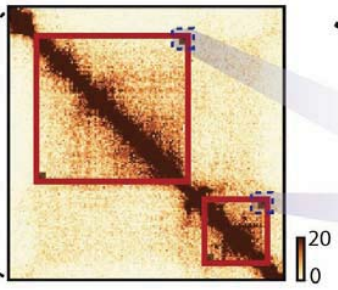
Compartment A/B



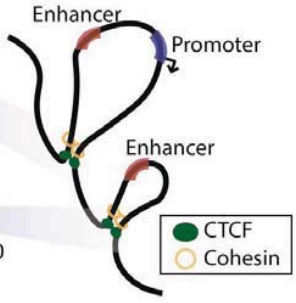
Individual chromosome



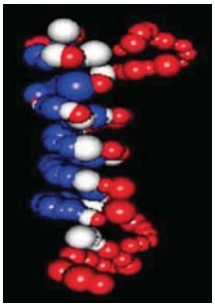
TAD (Topologically associating domains)



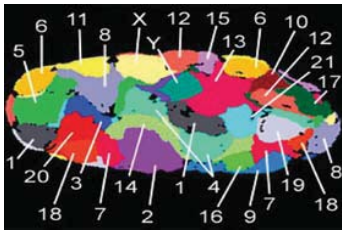
E-P interactions



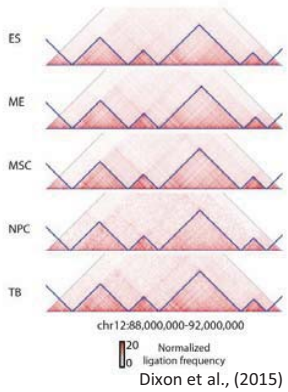
Kim et al., 2019



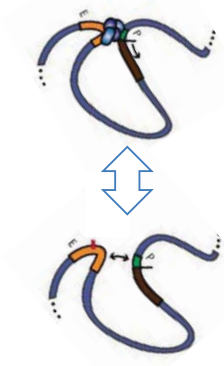
Hu et al., (2013)



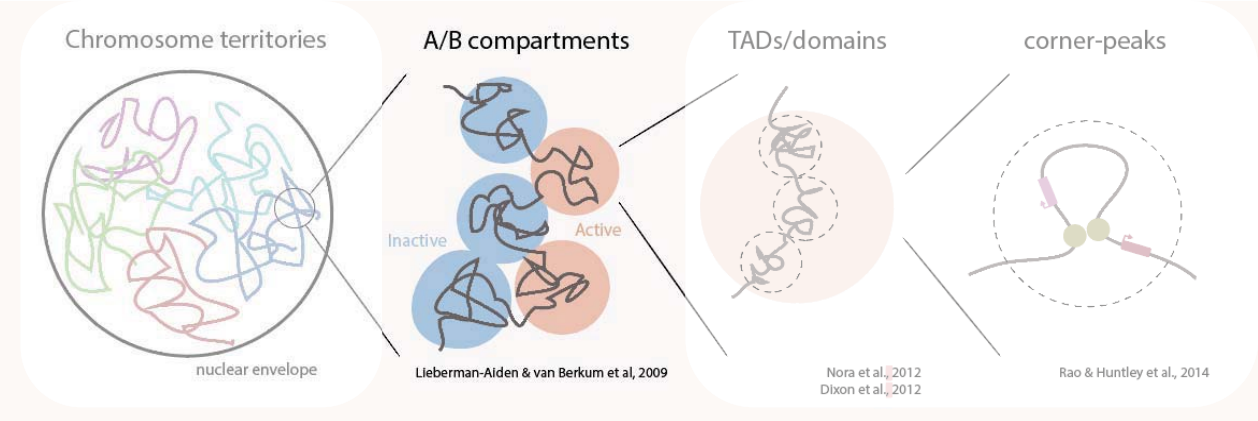
Bolzer et al., (2005)



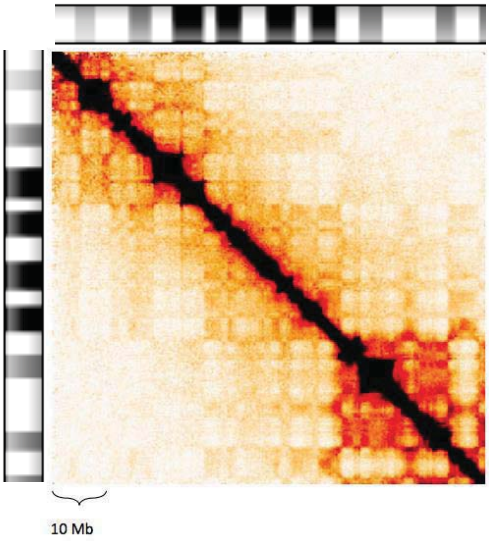
Dixon et al., (2015)

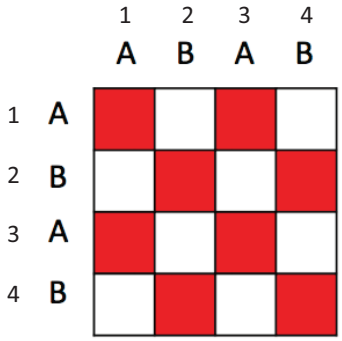


Jung et al., (2019)



Spatial compartmentalization of 3D genome



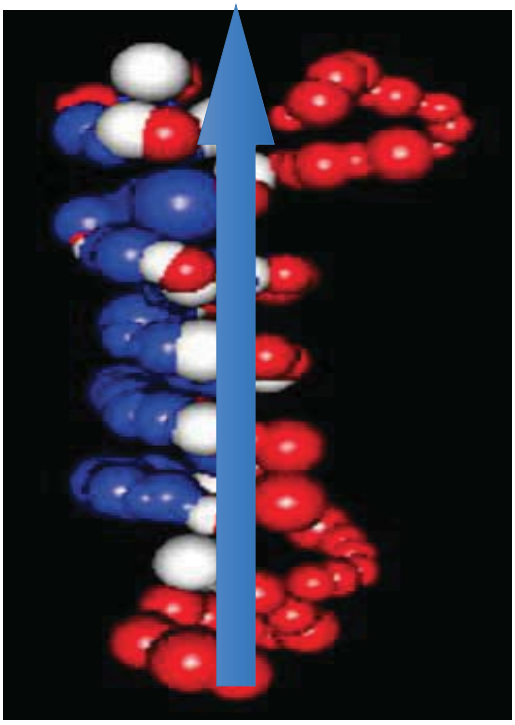


- What does a plaid pattern indicate for?
  - Higher interaction between fragment 1 and 3 and between fragment 2 and 4

- What is a biological meaning of the presence of a plaid pattern?
  - Genome can be compartmentalized into two parts (compartment A and B)

What is a major structural component?

Compartment B



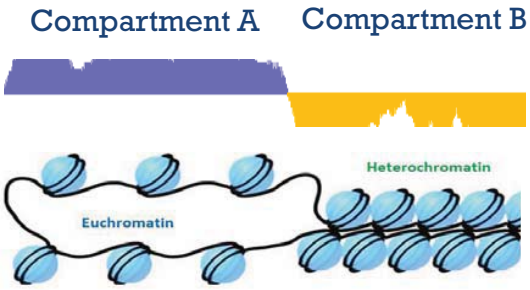
Compartment A

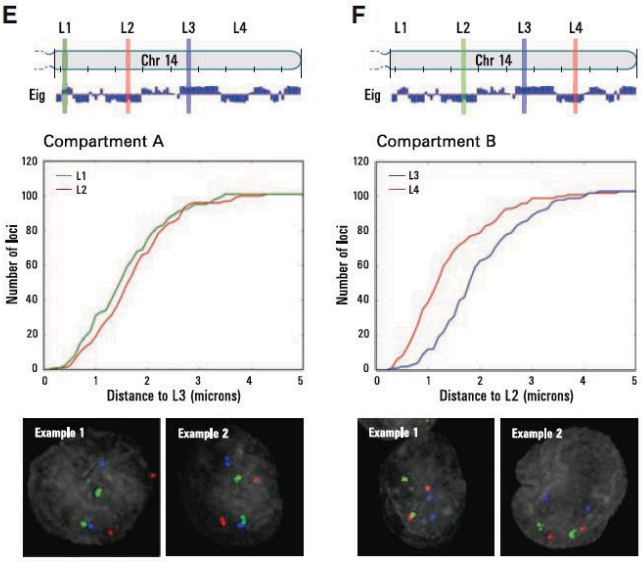
How can we systematically compartmentalize the 3D genome structure into two parts?

Two major compositions of chromatin structure: Compartment A/B

How does compartment A/B affect spatial genome organization?

The loci in the same compartment showed spatial proximity

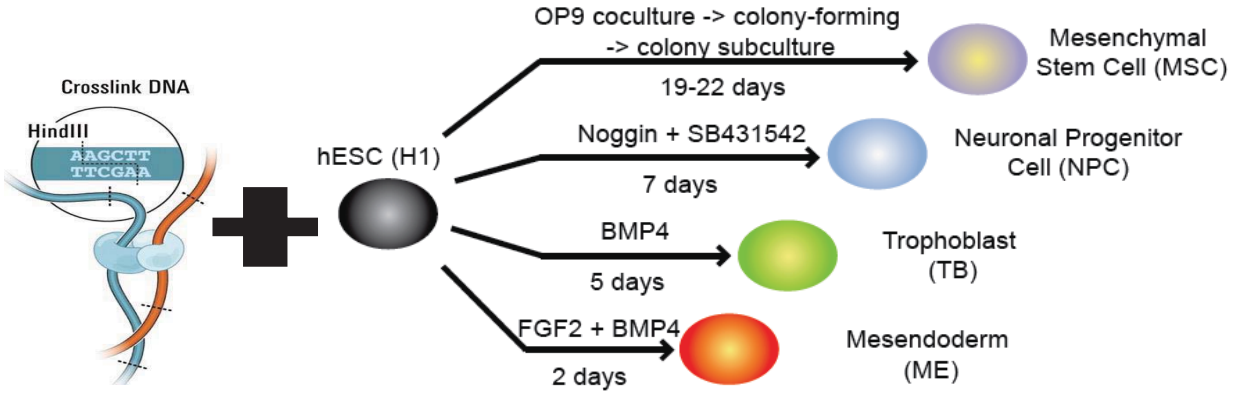




Compartment A/B dynamics during stem cell differentiation

Is compartment A/B cell-type specific?

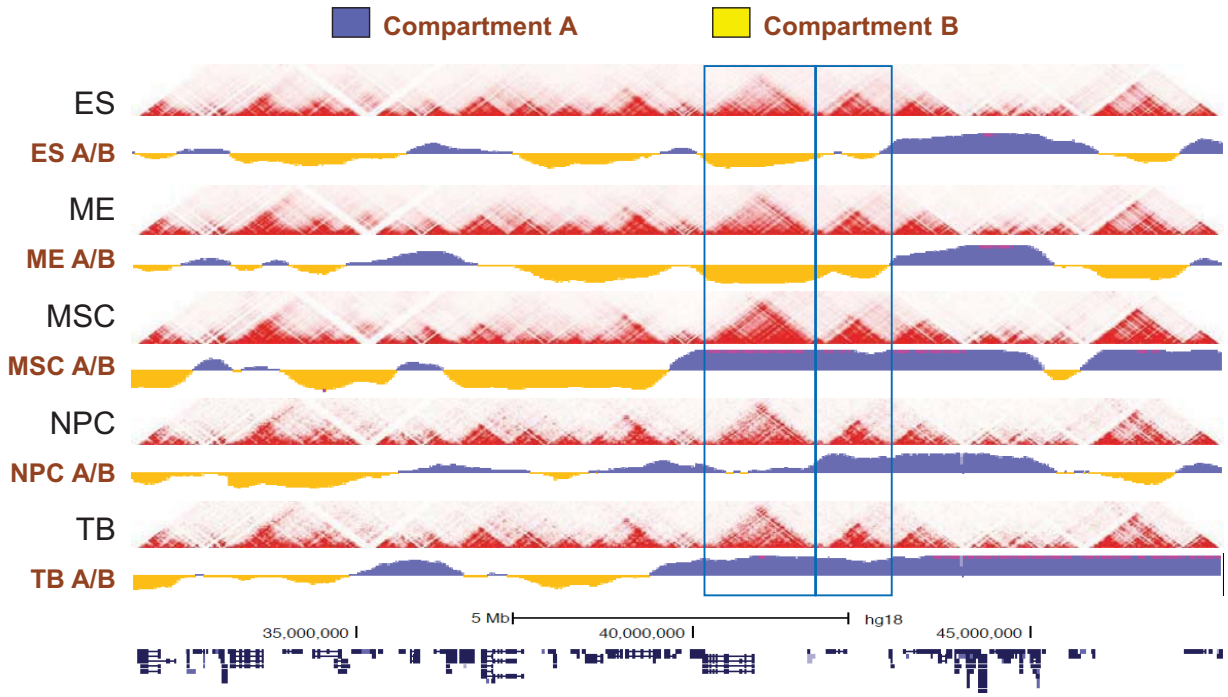
(How can we design a test experiment?)



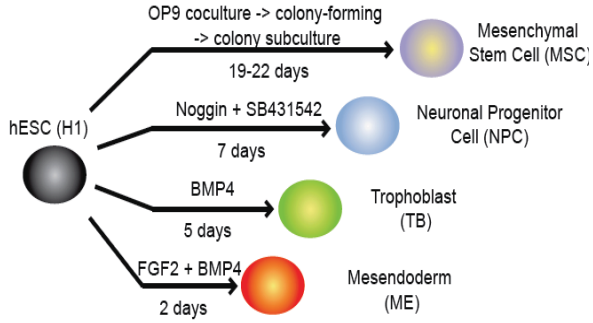
Perform Hi-C experiment

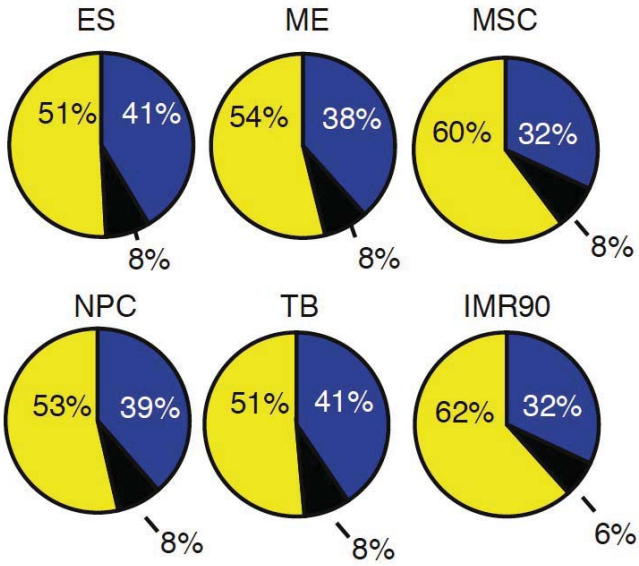
Dixon, JR., Jung, I., et al., Nature (2015)

Compartment A/B patterns are highly dynamic

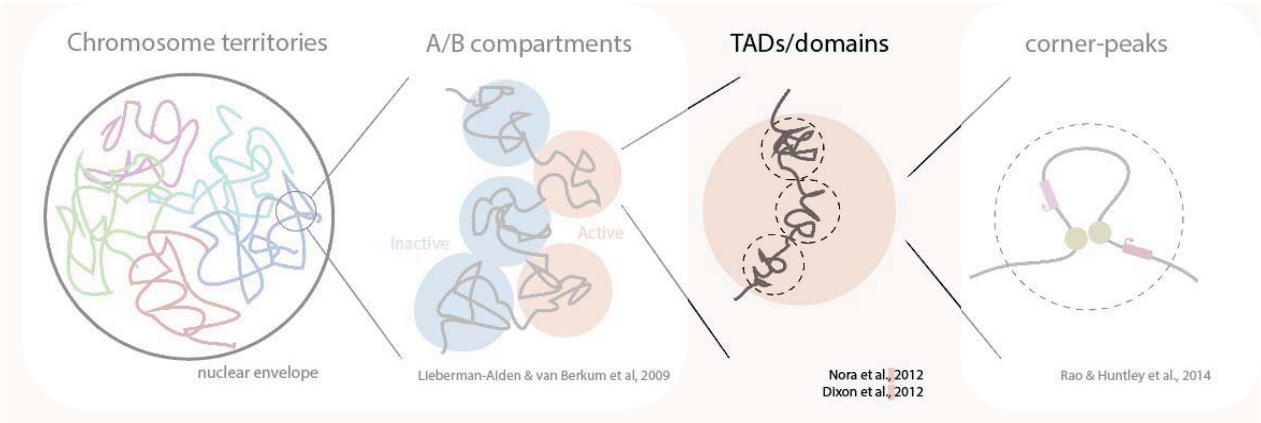


Fraction of compartment A/B in each cell-type

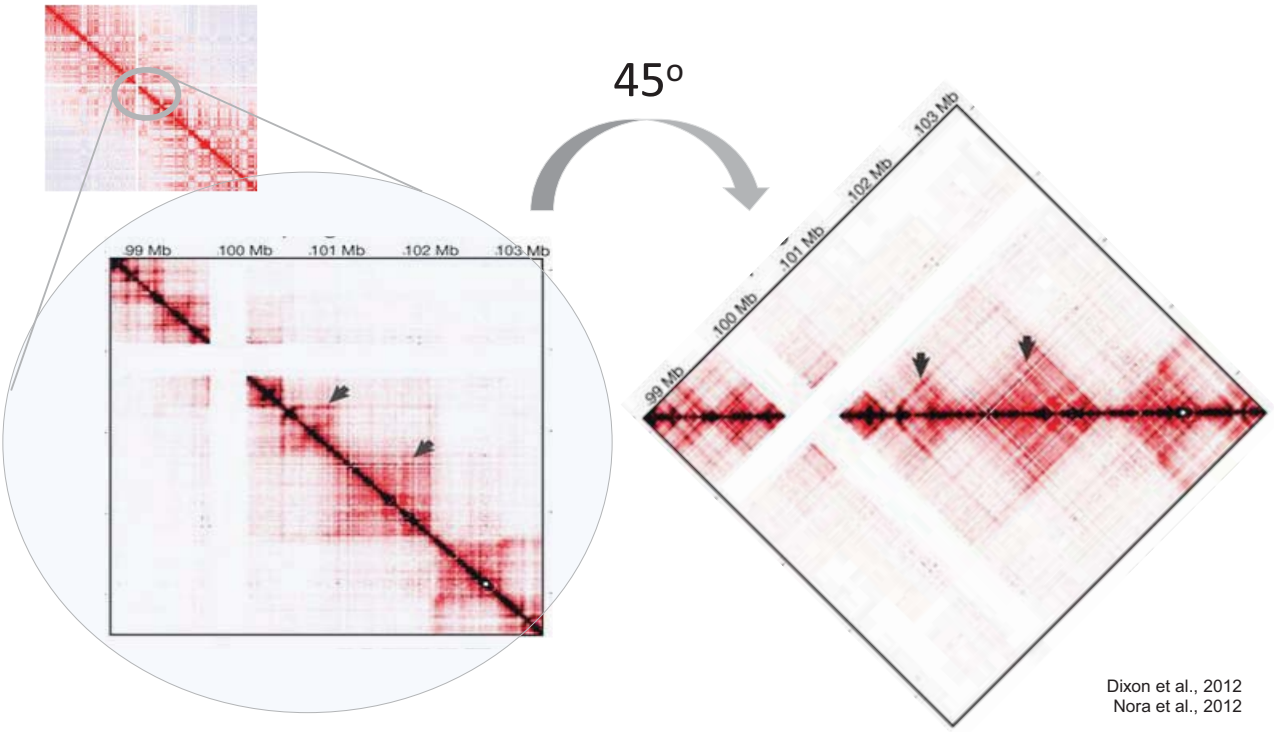




Fraction of genome marked as compartment A (blue) and B (yellow)

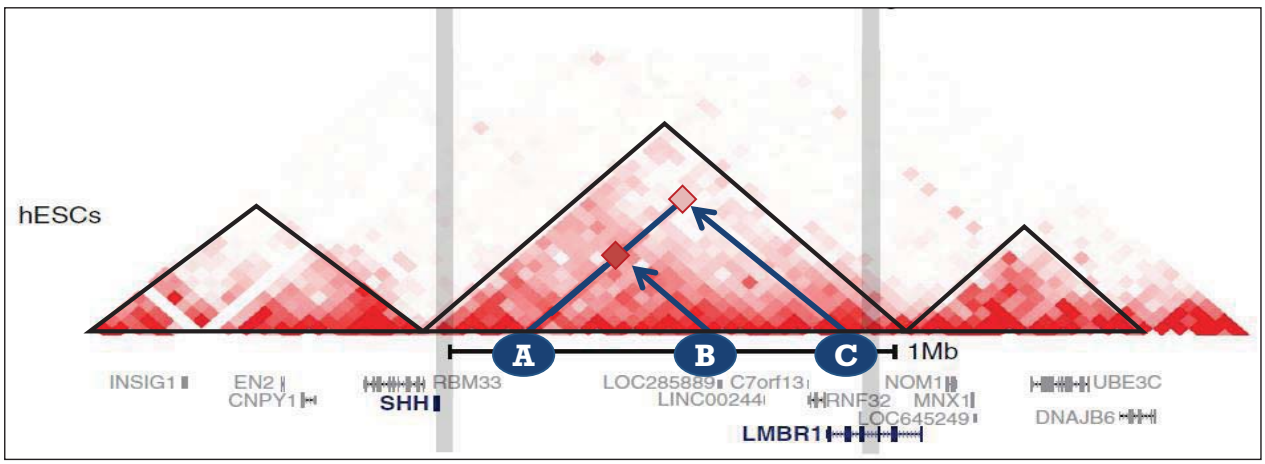


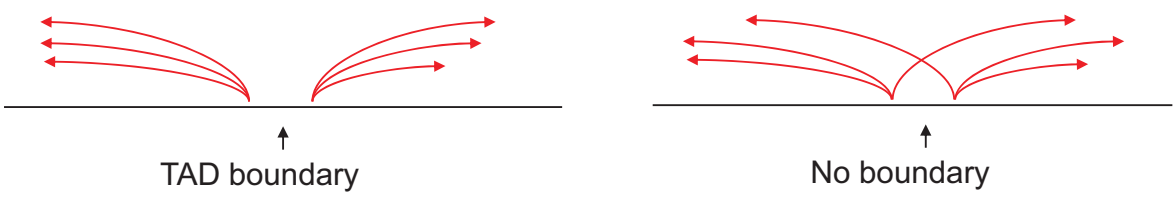
Topologically Associating Domains (TADs)



Dixon et al., 2012
Nora et al., 2012

Topologically Associating Domains (TADs)

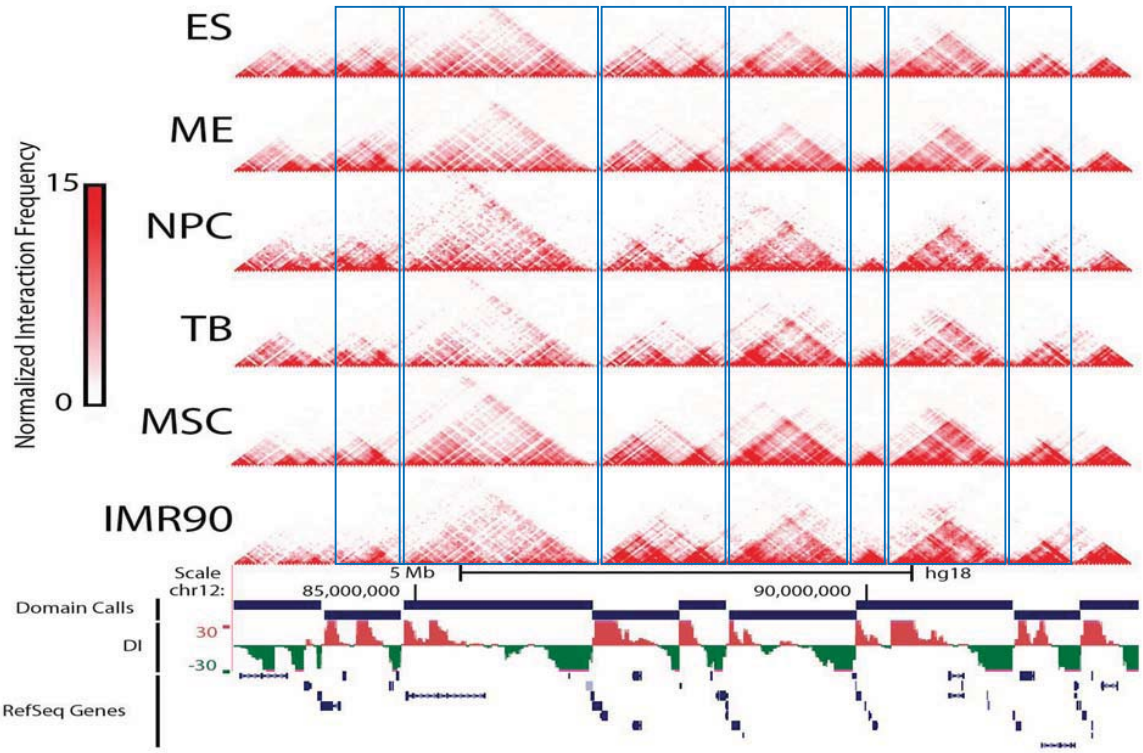




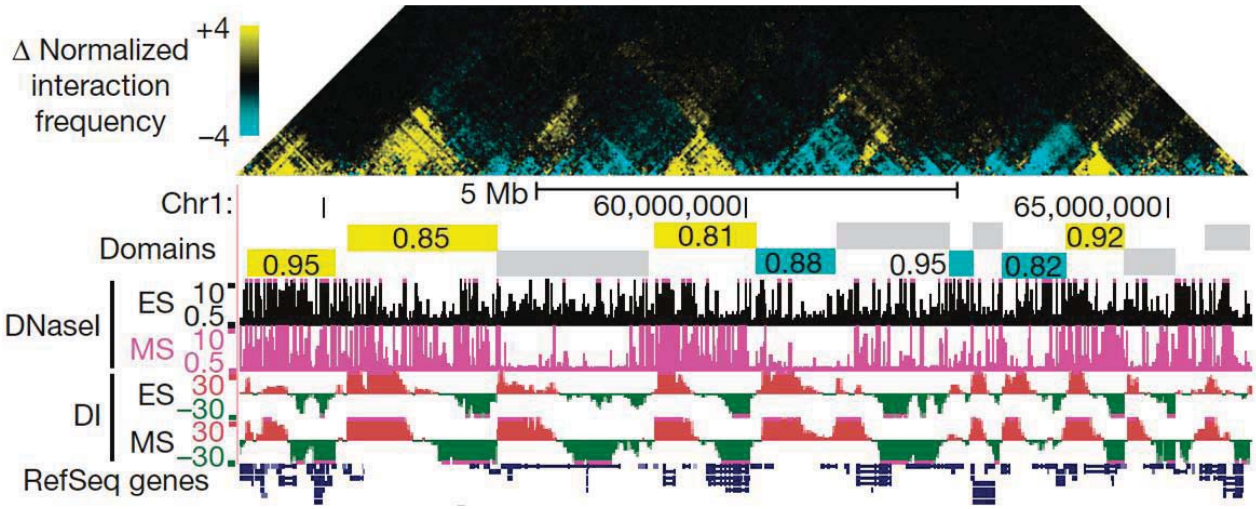
TAD boundary

No boundary

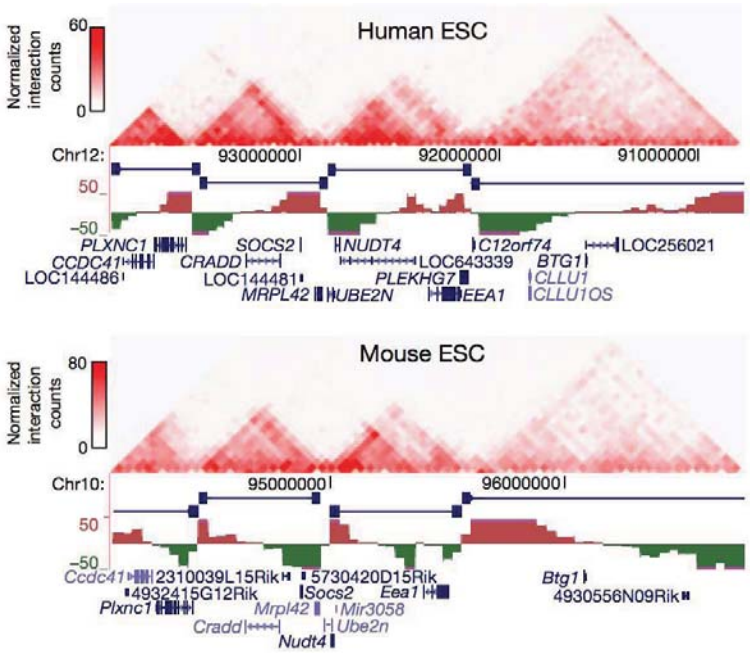
TAD boundaries are well maintained during differentiation

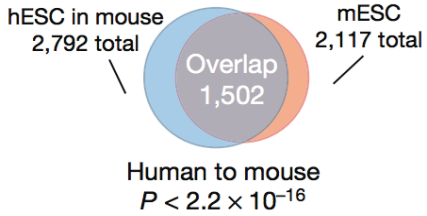


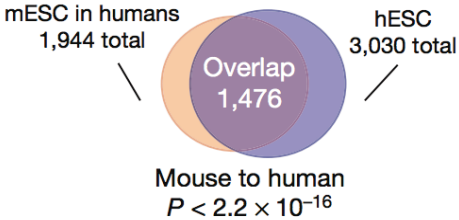
TAD-wise interaction changes during cellular differentiation



TAD boundaries are evolutionarily well conserved

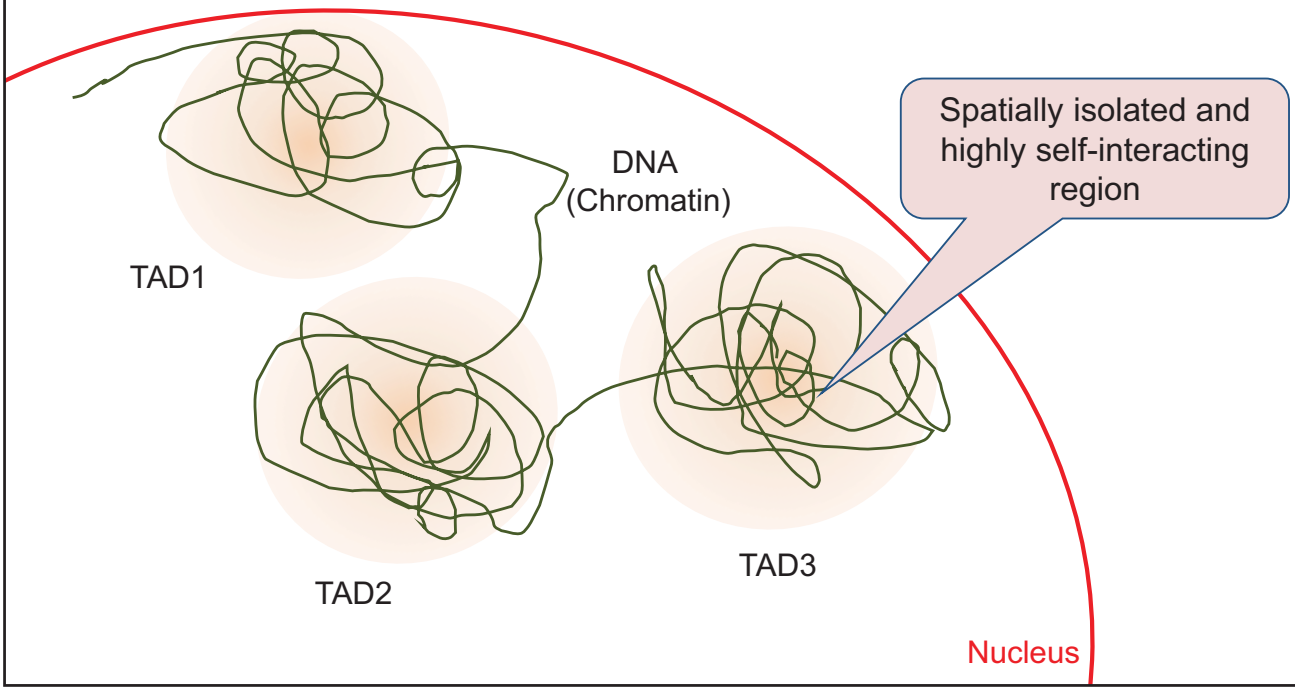




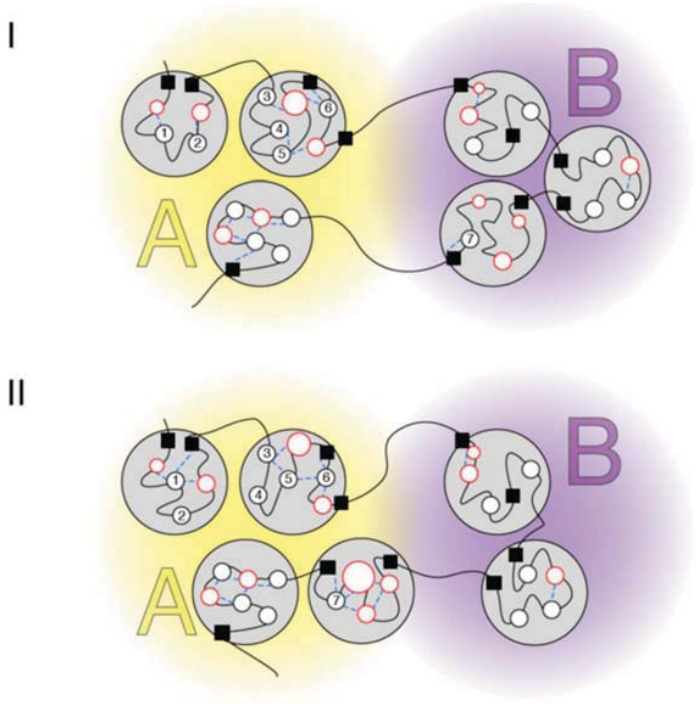


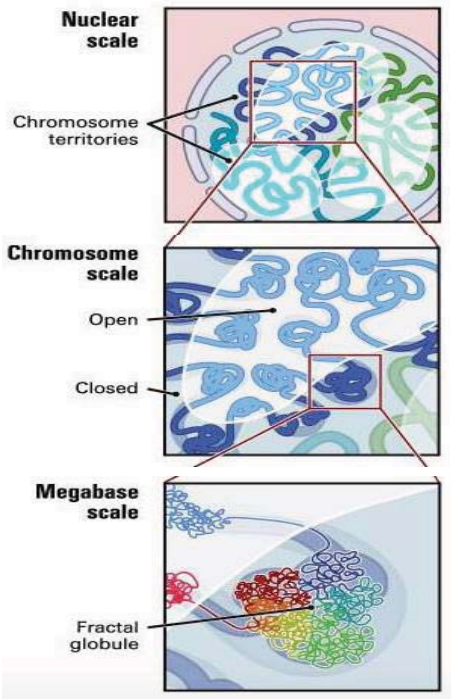
TAD is a basic unit of 3D chromatin structure

1. The human genome is organized into 2000~3000 TADs
2. TAD boundaries are well maintained during cellular differentiation and evolution
3. However, within TAD interactions are dynamic in cell-type specific manner



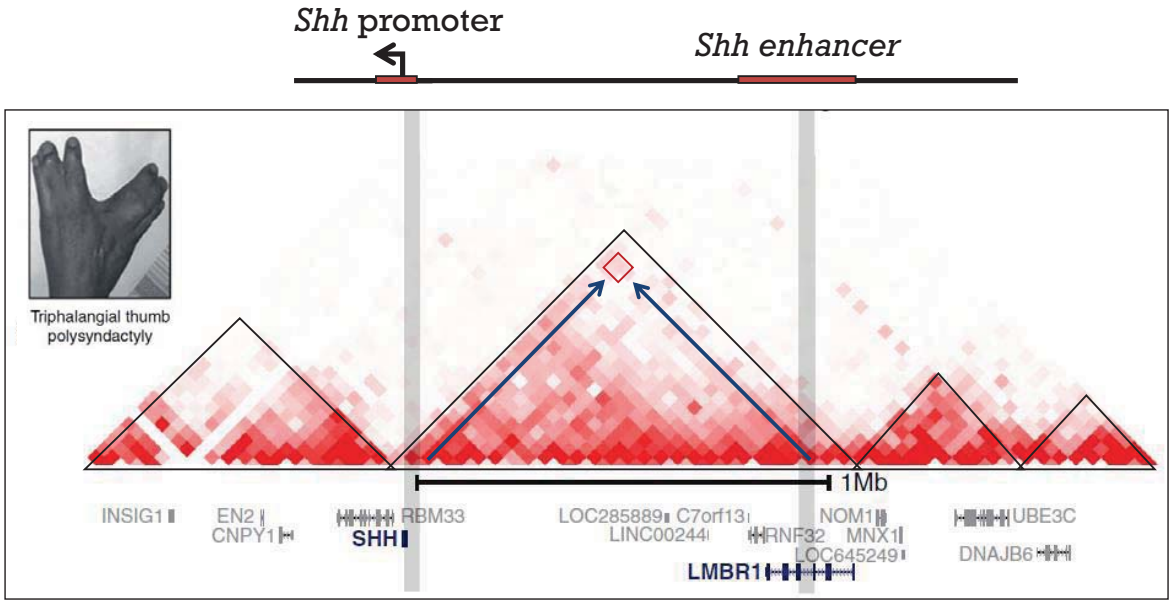
What is a relationship between TAD and Compartment A/B?





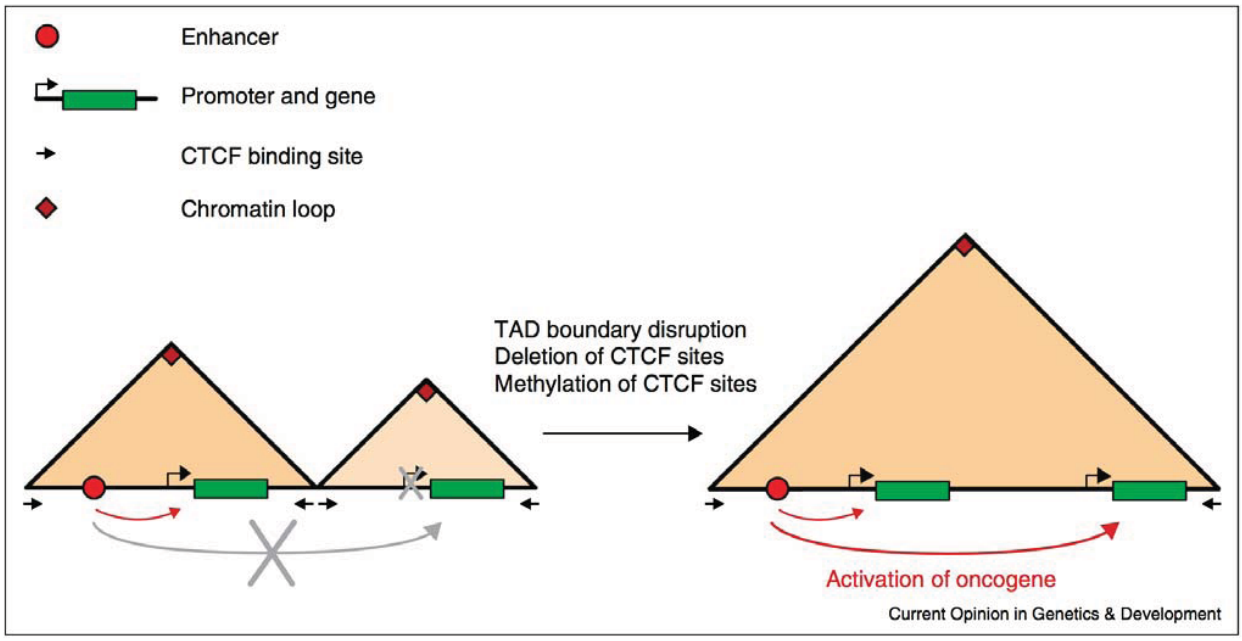
What is a functional role of TADs?

TAD boundary restricts long-range enhancer controls



From Dixon et al, Nature (2012) and Smallwood et al, Current Opinion Cell Biology (2013)

TAD boundary disruption as oncogenic driver – Model 1



Activation of proto-oncogenes by disruption of TAD boundary

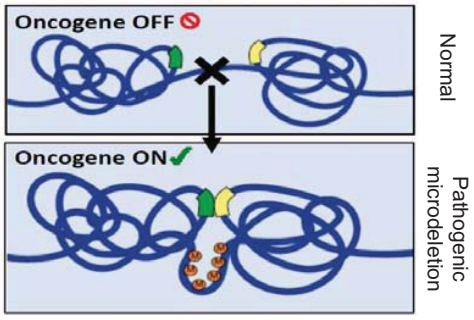
CANCER

Activation of proto-oncogenes by disruption of chromosome neighborhoods

Denes Hnisz,[1]* Abraham S. Weintraub,[1,2]* Daniel S. Day,[1] Anne-Laure Valton,[3] Rasmus O. Bak,[4] Charles H. Li,[1,2] Johanna Goldmann,[1] Bryan R. Lajoie,[3] Zi Peng Fan,[1,5] Alla A. Sigova,[1] Jessica Reddy,[1,2] Diego Borges-Rivera,[1,2] Tong Ihn Lee,[1] Rudolf Jaenisch,[1,2] Matthew H. Porteus,[4] Job Dekker,[3,6] Richard A. Young[1,2]†

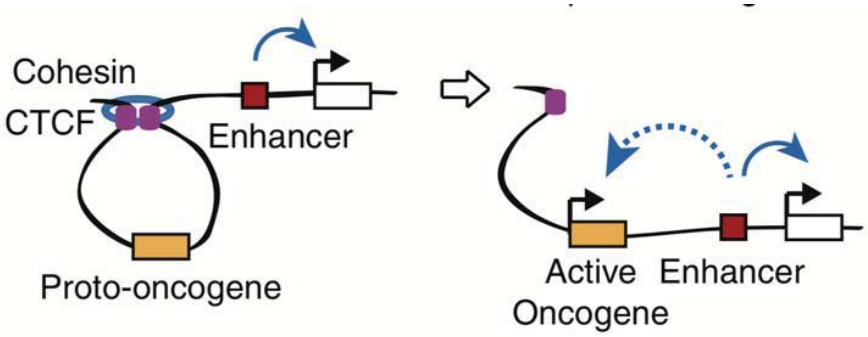
Oncogenes are activated through well-known chromosomal alterations such as gene fusion, translocation, and focal amplification. In light of recent evidence that the control of key genes depends on chromosome structures called insulated neighborhoods, we investigated whether proto-oncogenes occur within these structures and whether oncogene activation can occur via disruption of insulated neighborhood boundaries in cancer cells. We mapped insulated neighborhoods in T cell acute lymphoblastic leukemia (T-ALL) and found that tumor cell genomes contain recurrent microdeletions that eliminate the boundary sites of insulated neighborhoods containing prominent T-ALL proto-oncogenes. Perturbation of such boundaries in nonmalignant cells was sufficient to activate proto-oncogenes. Mutations affecting chromosome neighborhood boundaries were found in many types of cancer. Thus, oncogene activation can occur via genetic alterations that disrupt insulated neighborhoods in malignant cells.

TAD boundary disruption induces oncogene activation



Hnisz et al., 2016 (Science)

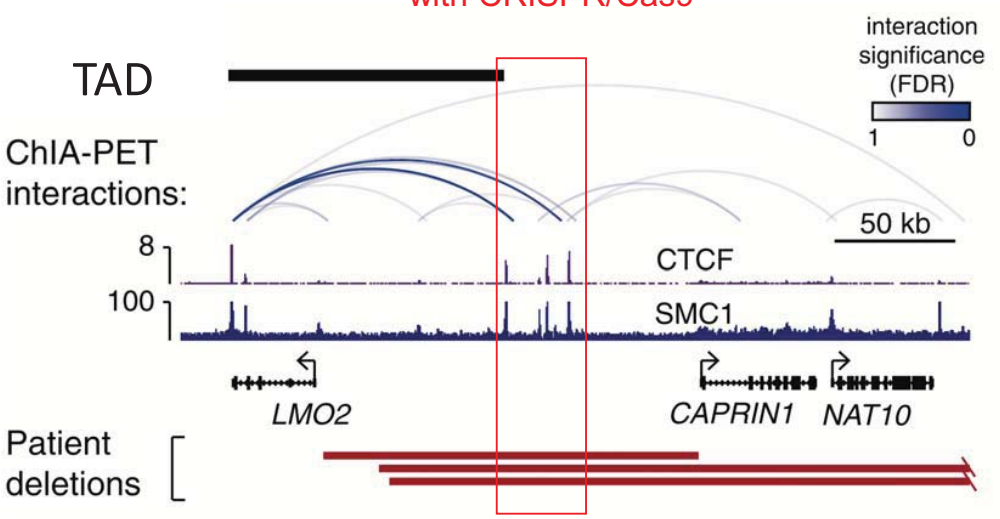
T-cell acute lymphoblastic leukemia (T-ALL)



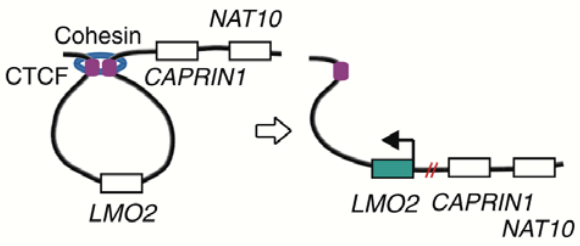
Can disruption of TAD boundary (TAD fusion) activate proto–oncogenes through enhancer–hijacking?

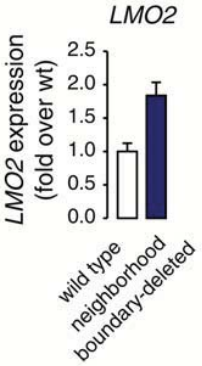
Disruption of TAD boundary by CRISPR/Cas9

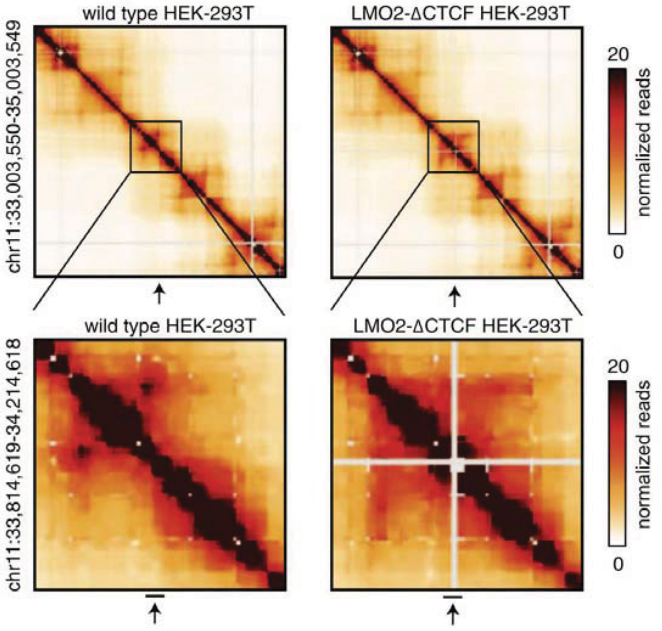
Disruption of insulated neighborhood
with CRISPR/Cas9

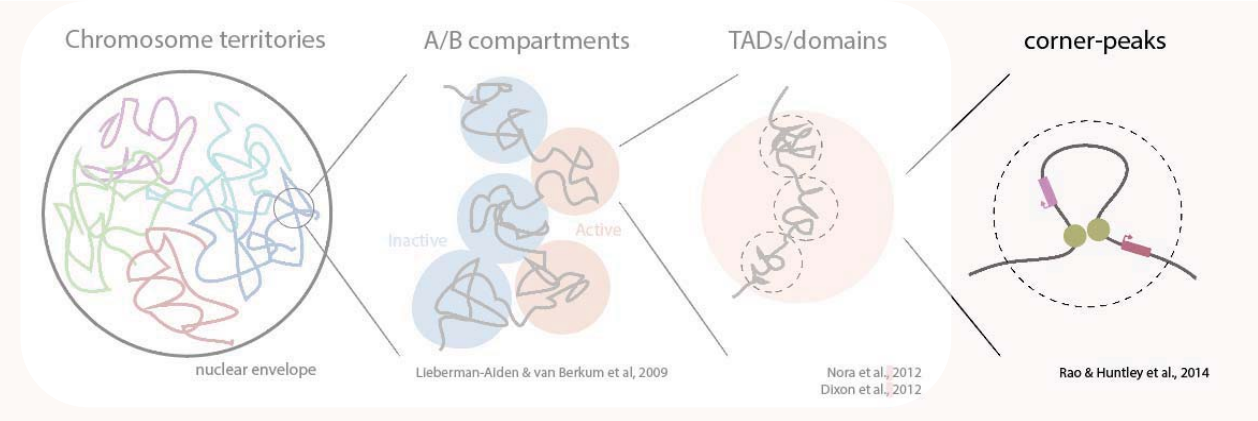


Disruption of TAD boundary activates LMO2

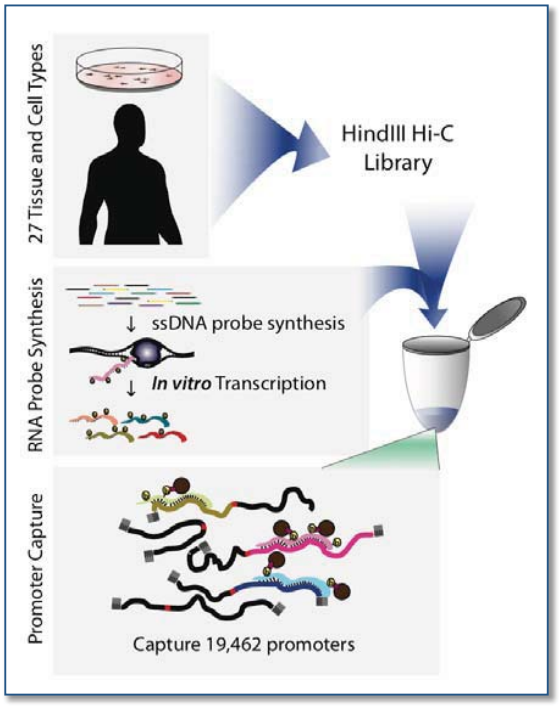


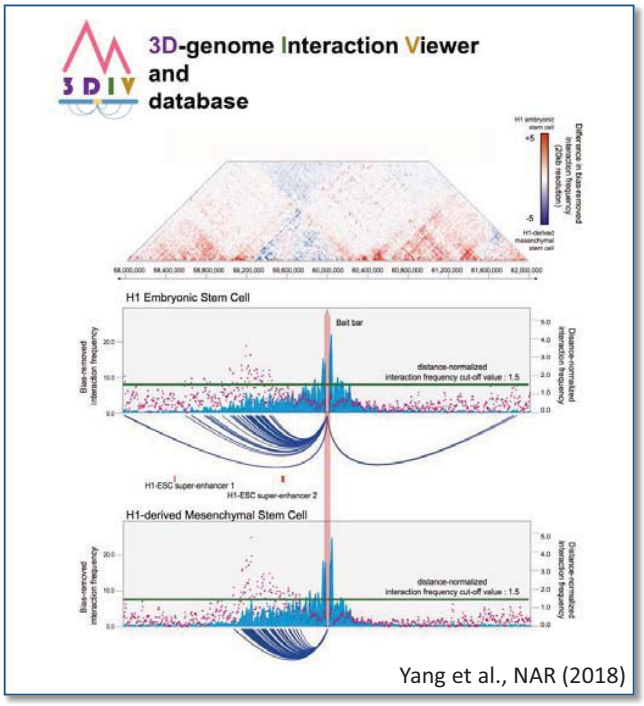






Promoter-capture Hi-C: Enhancer-promoter interaction maps



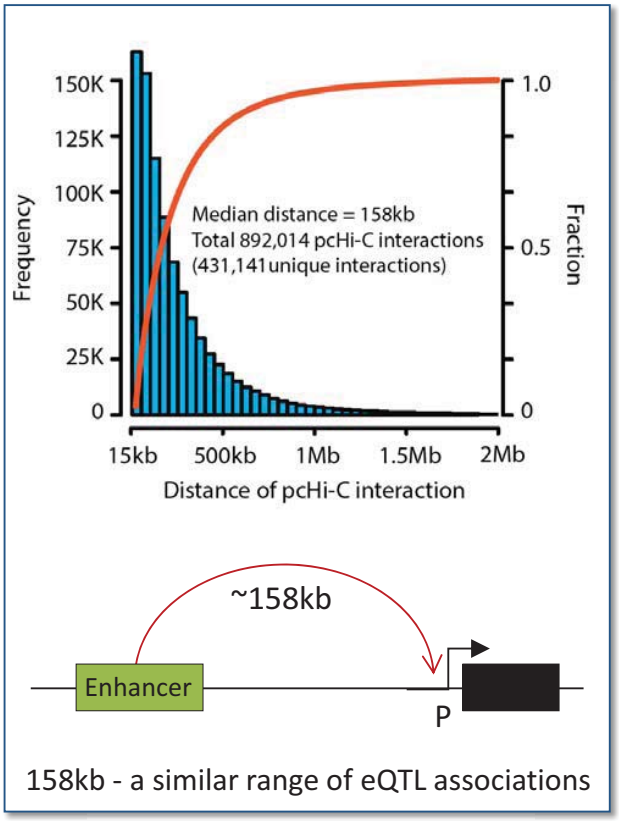


Yang et al., NAR (2018)

Jung et al., Nature Genetics (2019)

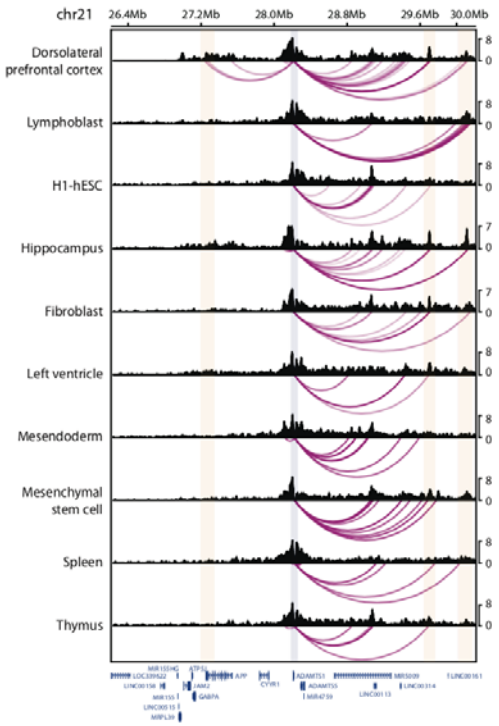
Basic principles of enhancer-promoter interactions

1. Enrichment of distal interactions



158kb - a similar range of eQTL associations

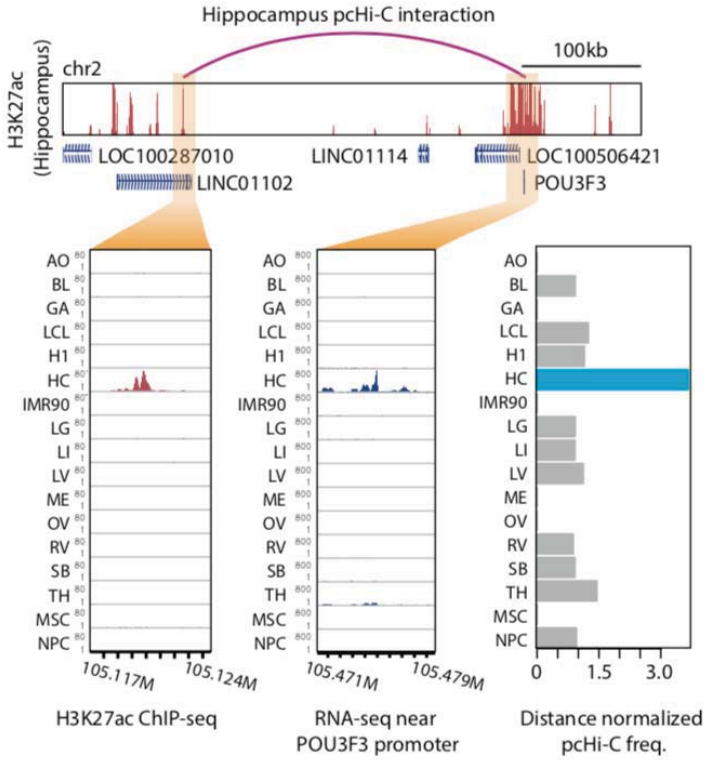
2. Interactions are tissue-specific

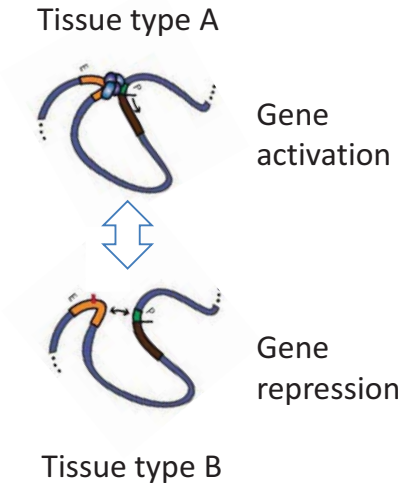


13~45% of pcHi-C interactions are unique

Basic principles of enhancer-promoter interactions

3. E-P interactions correlate with tissue-specific gene expression





Summary

- Non-coding sequences are occupied by regulatory elements
- Regulatory elements can control target gene expression in large genomic distance
- The genome is organized into multiple-layers
- TAD is a basic structural and functional unit of 3D chromatin structure
- Disruption of TAD may potentiate disease-specific gene expression
- Long-range enhancer-promoter interactions are critical in cell/tissue-specific gene expression

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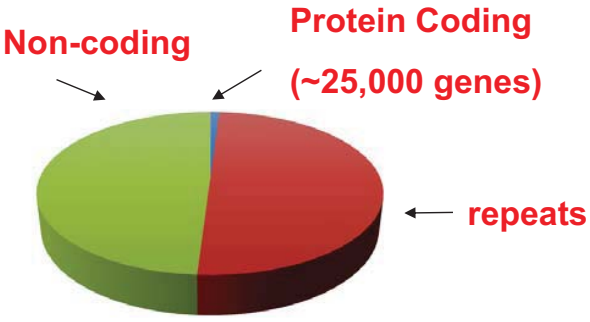
KSBi-BIML 2024

(Single-cell) 3D Epigenome Data Analysis

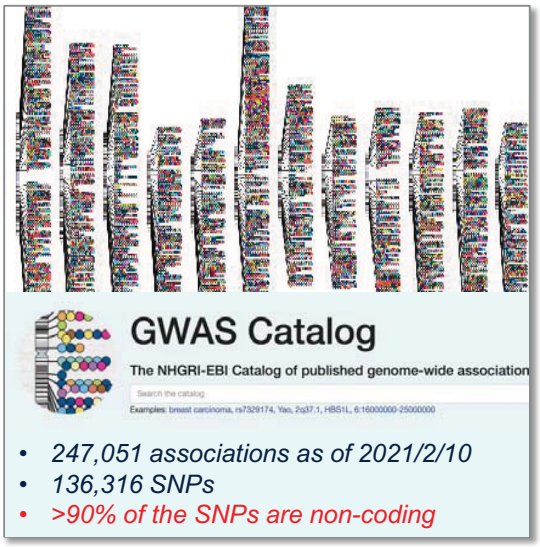
정인경(KAIST)

Contents

3D genome: a new way to the human genome

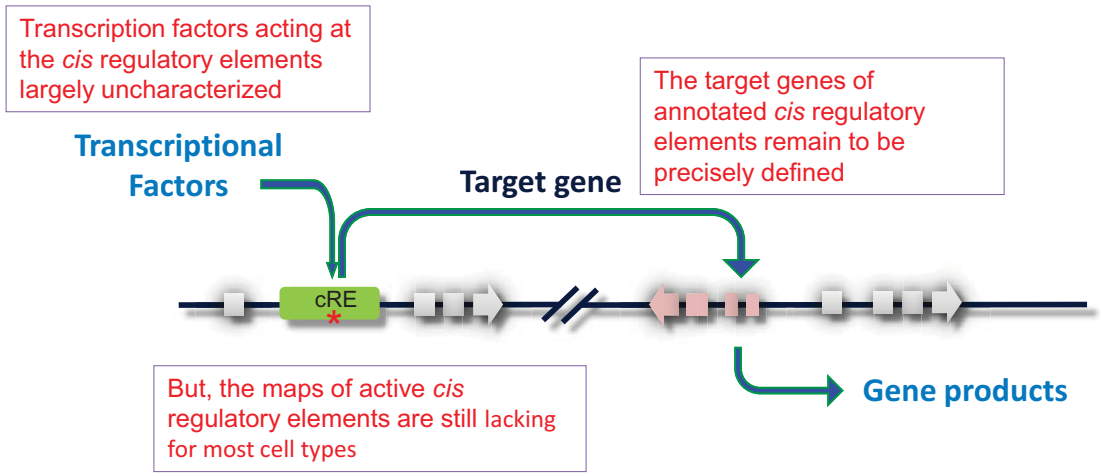


$3x10^9$ basepairs

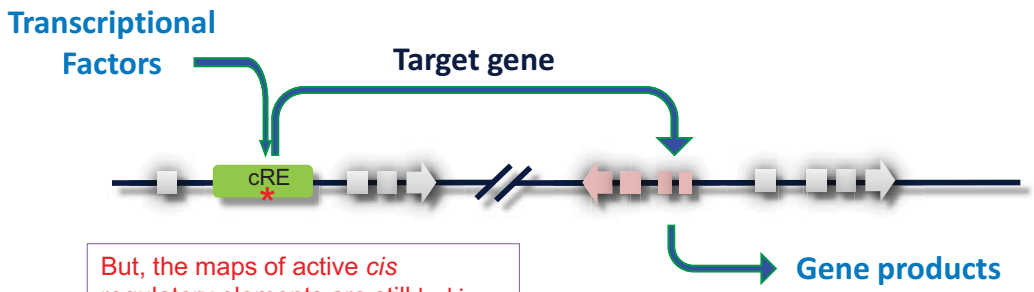


https://www.ebi.ac.uk/gwas/

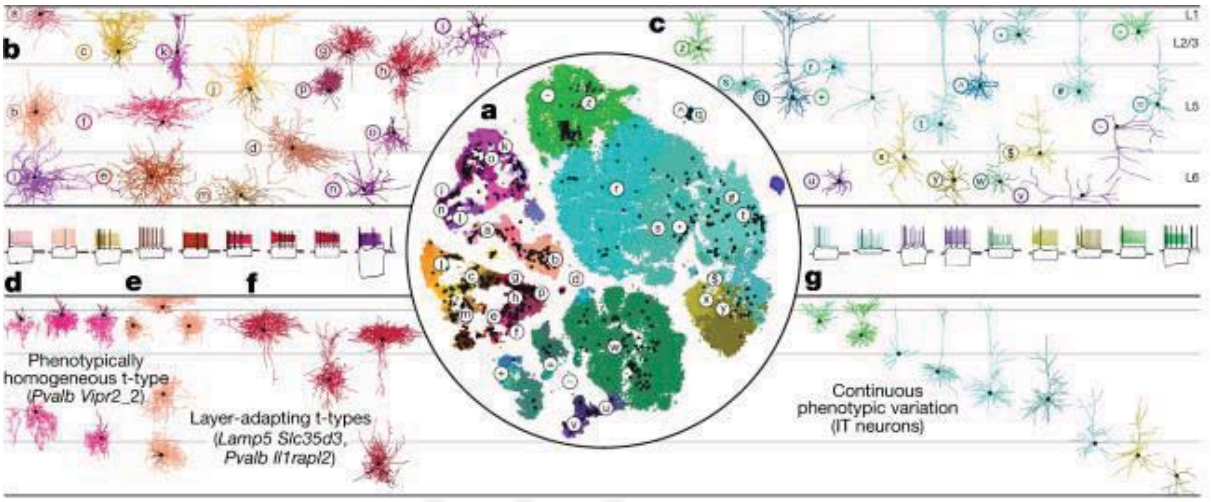
Hypothesis: noncoding variants disrupt *cis* regulatory element s active in disease relevant cell types



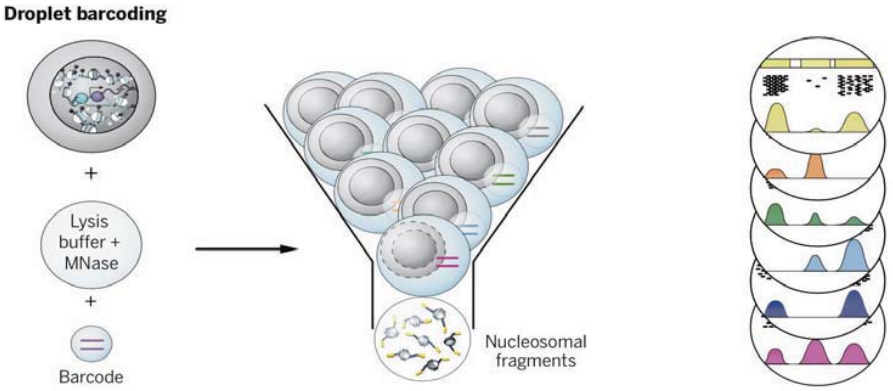
cRE: cis regulatory elements



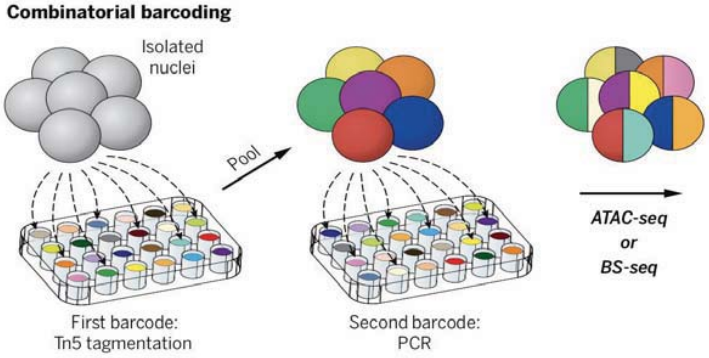
But, the maps of active *cis* regulatory elements are still lacking for **most cell types**



Single-cell Epigenomics

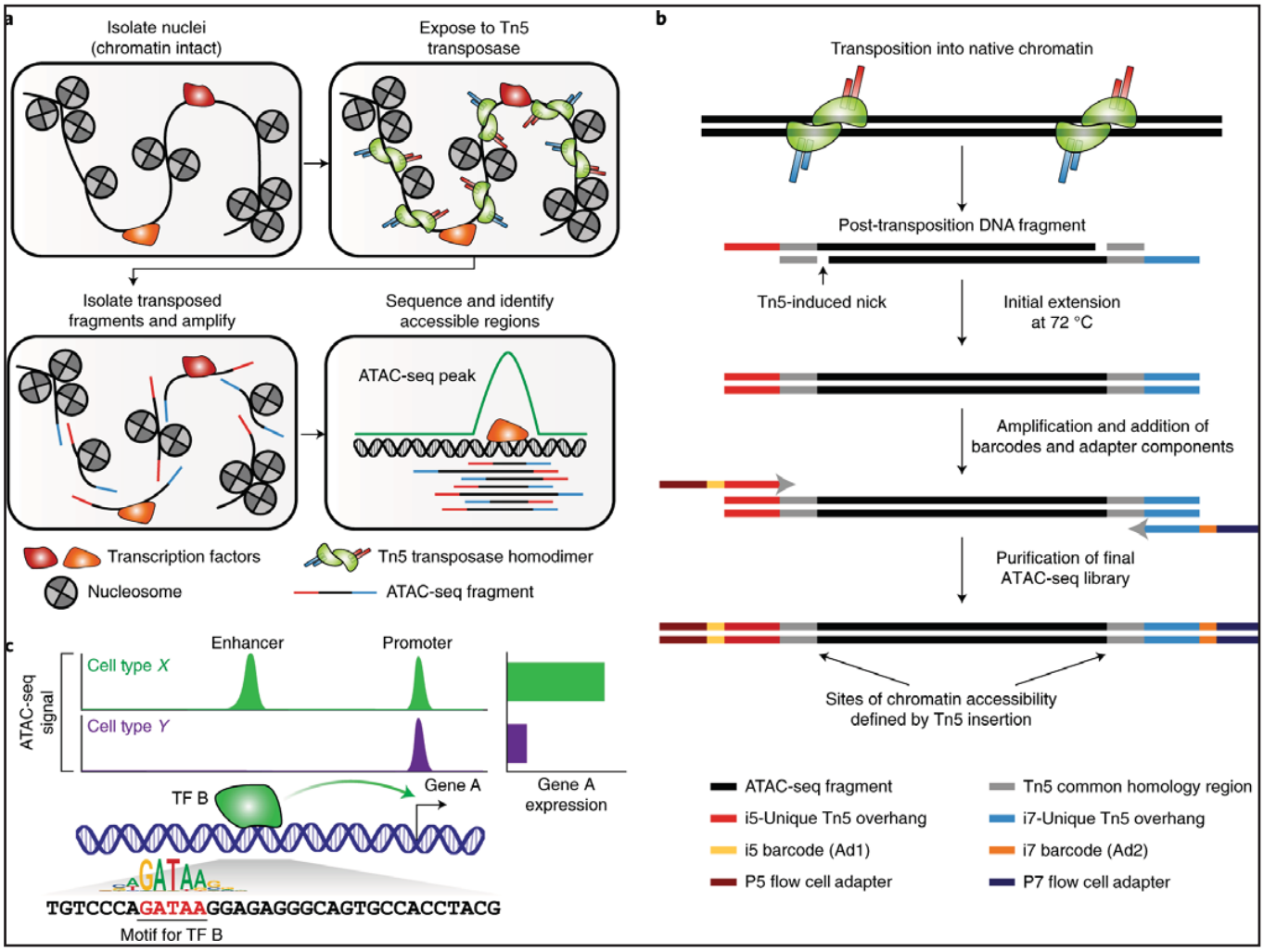


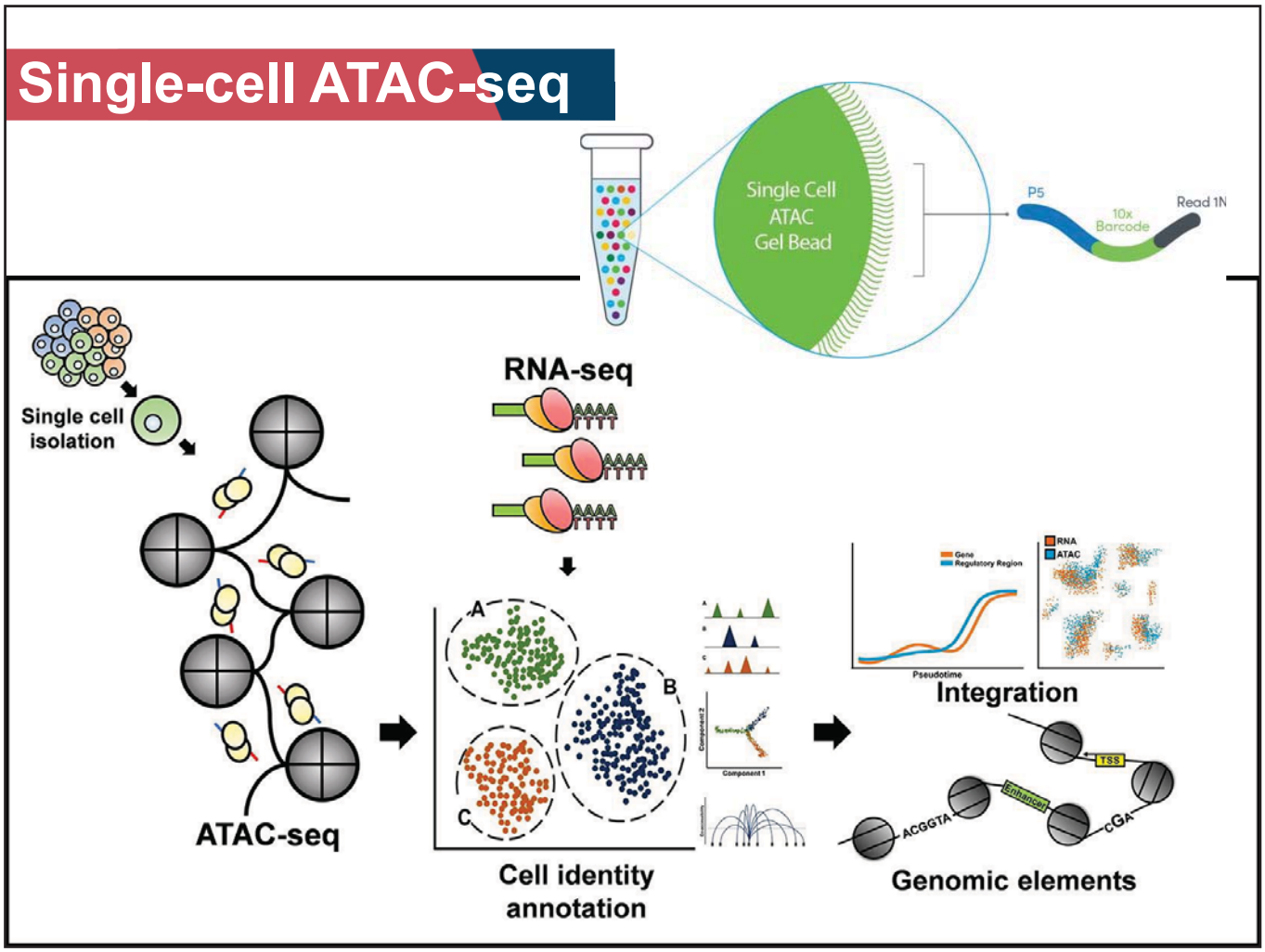
- Commercially available
- Robust
- scATAC-seq
- sc-RNA/ATAC Multiome



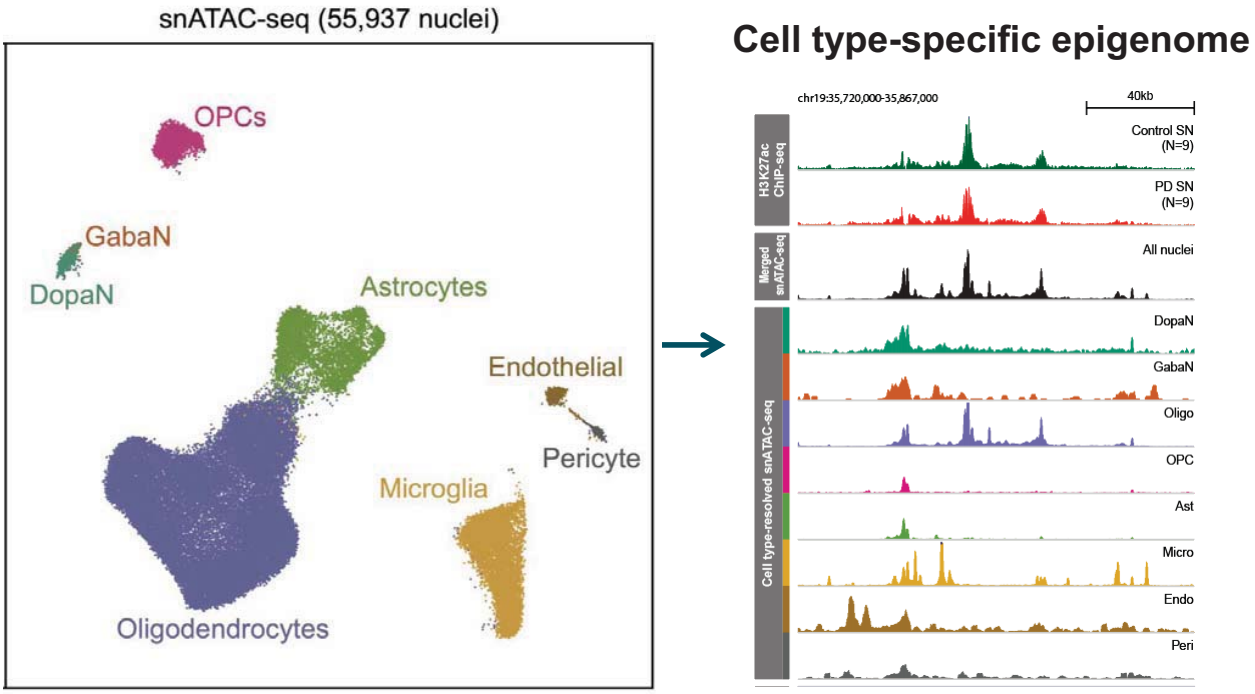
- Customizable
- Highly scalable (10^4-10^6)
- RNA, ATAC, HiC, mC, etc
- Mutil-omics
- No specialized Instrument

Modified from Kelsey, Stegle and Reik, *Science* 358, 69 (2017)

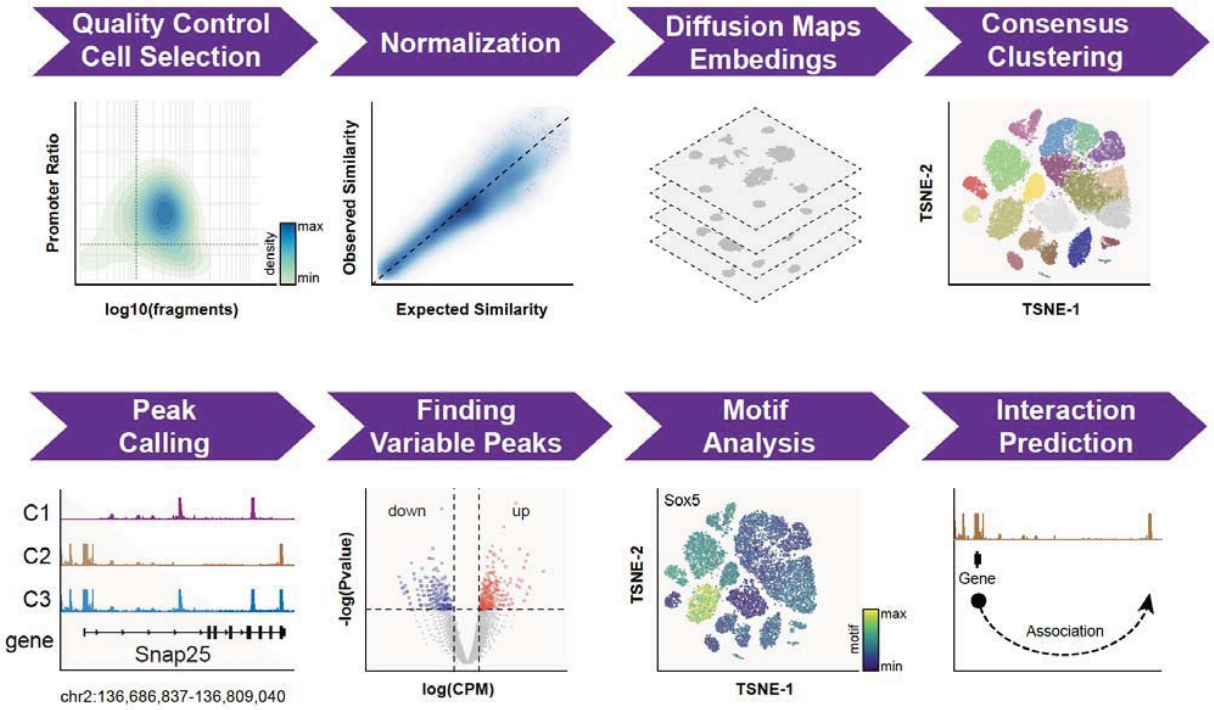

a
Isolate nuclei
(chromatin intact)
Expose to Tn5
transposase
Isolate transposed
fragments and amplify
Sequence and identify
accessible regions
ATAC-seq peak
Transcription factors
Nucleosome
Tn5 transposase homodimer
ATAC-seq fragment
b
Transposition into native chromatin
Post-transposition DNA fragment
Tn5-induced nick
Initial extension
at 72 °C
Amplification and addition of
barcodes and adapter components
Purification of final
ATAC-seq library
Sites of chromatin accessibility
defined by Tn5 insertion
ATAC-seq fragment
i5-Unique Tn5 overhang
i5 barcode (Ad1)
P5 flow cell adapter
Tn5 common homology region
i7-Unique Tn5 overhang
i7 barcode (Ad2)
P7 flow cell adapter
c
ATAC-seq
signal
Enhancer
Promoter
Cell type X
Cell type Y
Gene A
Gene A
expression
TF B
TGTCCCAGATAAGGAGAGGGCAGTGCCACCTACG
Motif for TF B


Single-cell ATAC-seq
Single Cell
ATAC
Gel Bead
P5
10x
Barcode
Read 1N
Single cell
isolation
RNA-seq
ATAC-seq
A
B
C
Cell identity
annotation
Integration
Pseudotime
RNA
ATAC
TSS
Enhancer
ACGGTA
CGA
Genomic elements

Defining PD brain epigenome using snATAC-seq

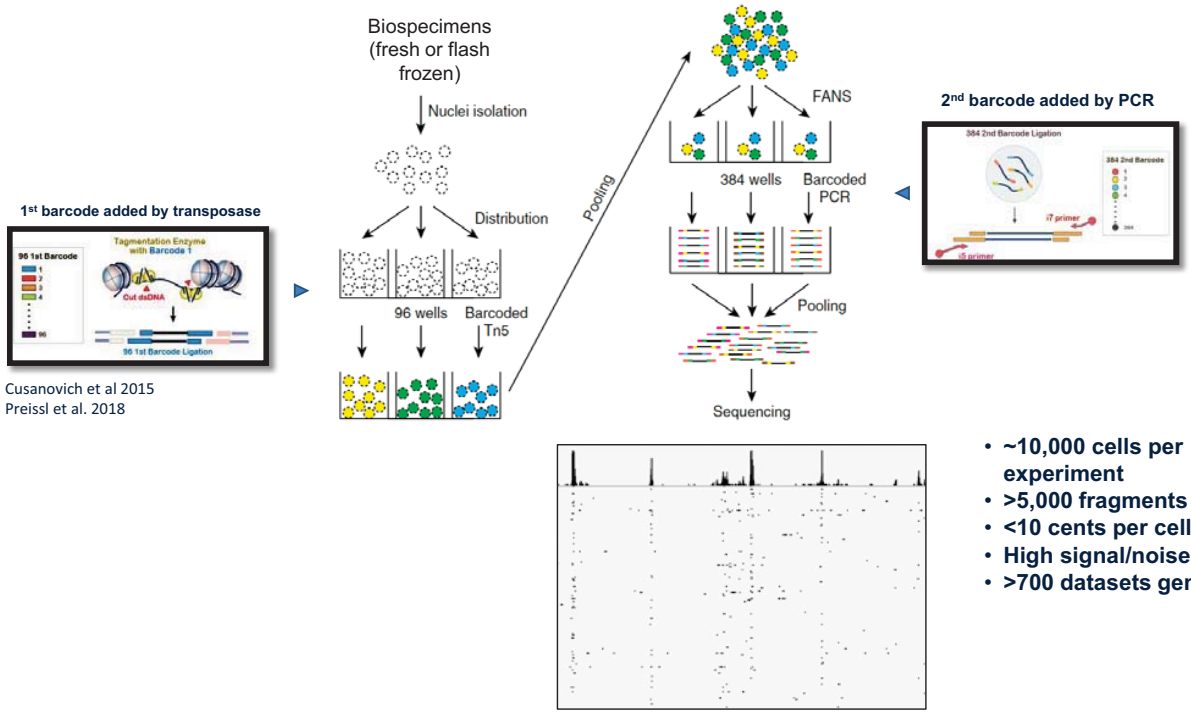


Single Nucleus Analysis Pipeline for ATAC-seq



Nature Communication (2021)

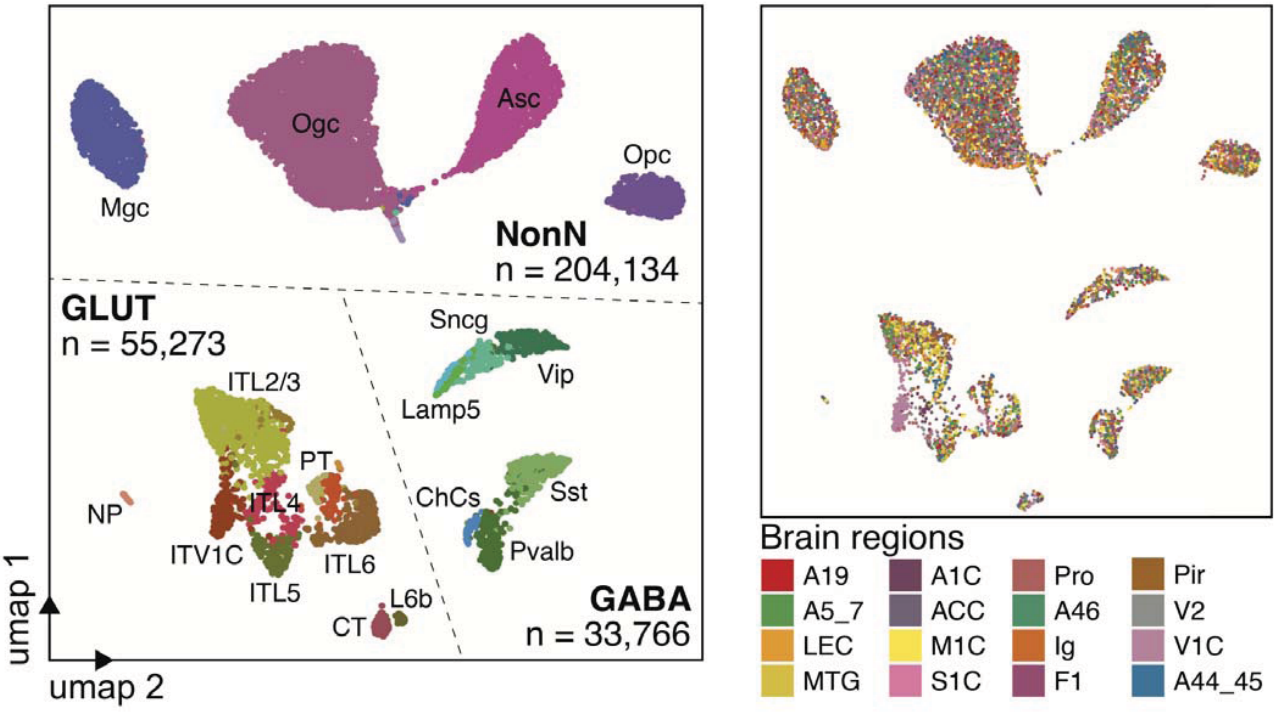
snATAC-seq by a combinatorial barcoding

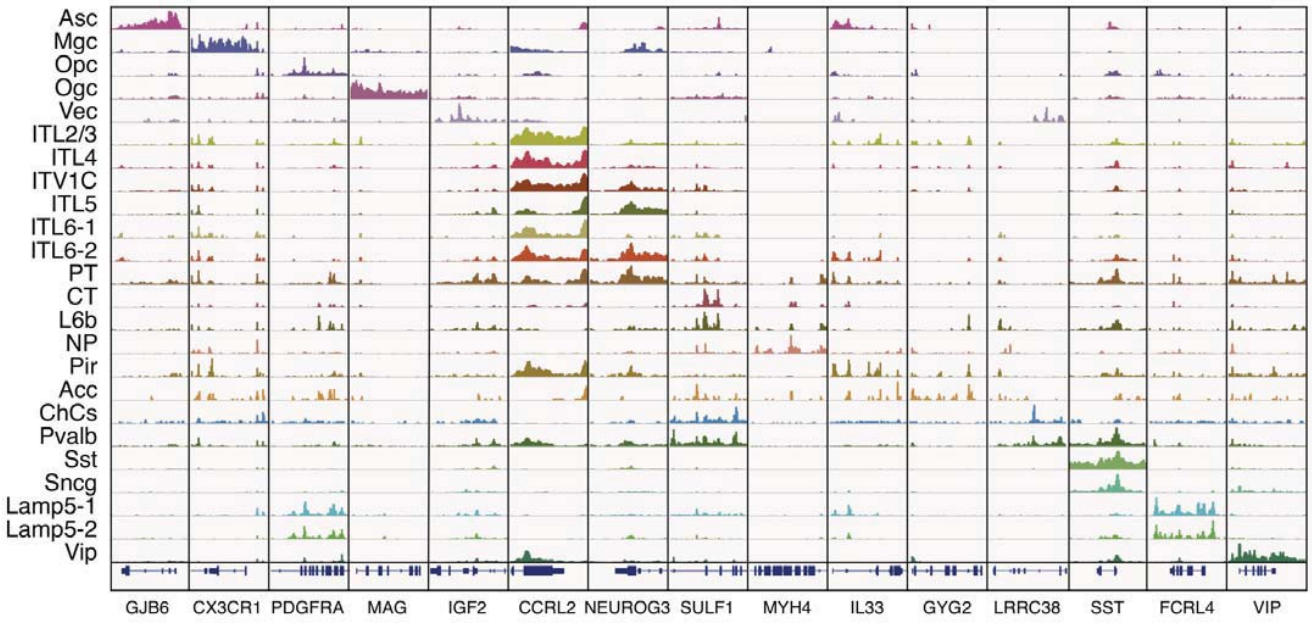


Cusanovich et al 2015
Preissl et al. 2018

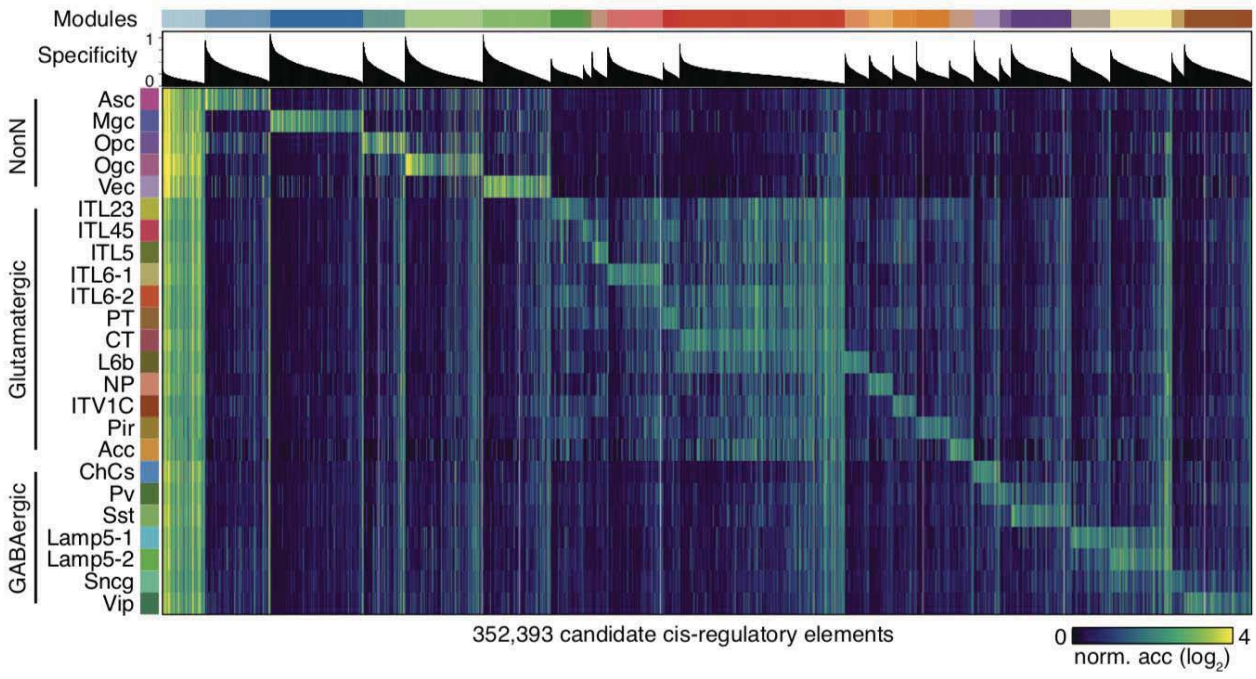
- ~10,000 cells per experiment
- >5,000 fragments per cell
- <10 cents per cell
- High signal/noise
- >700 datasets generated

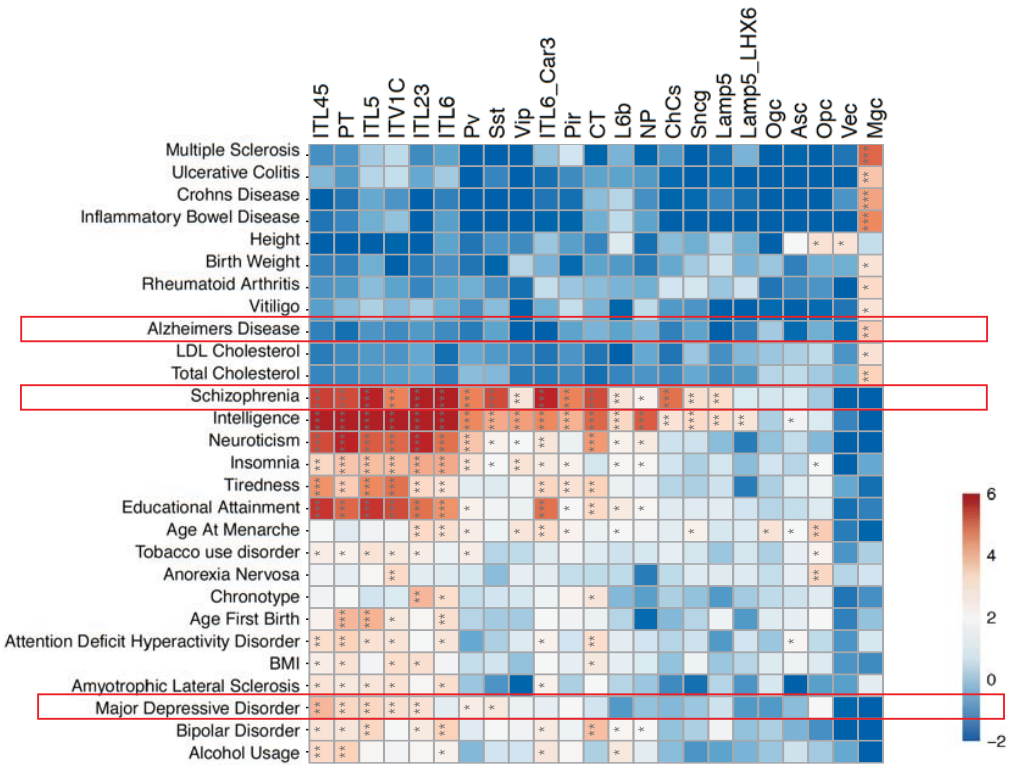
snATAC-seq in Human Cortex



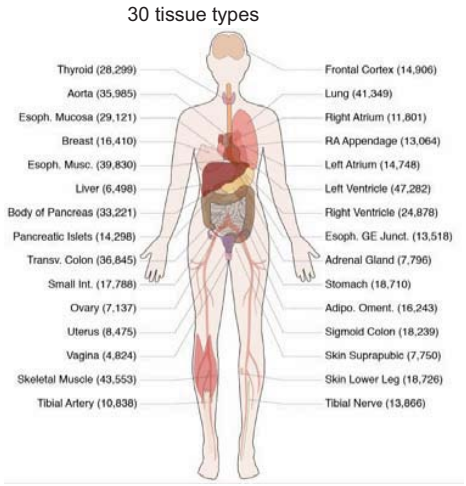


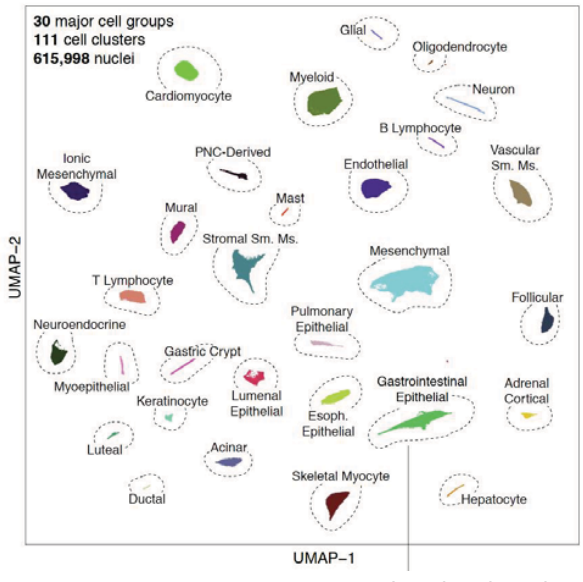
Annotation of CREs across human cortical cell types





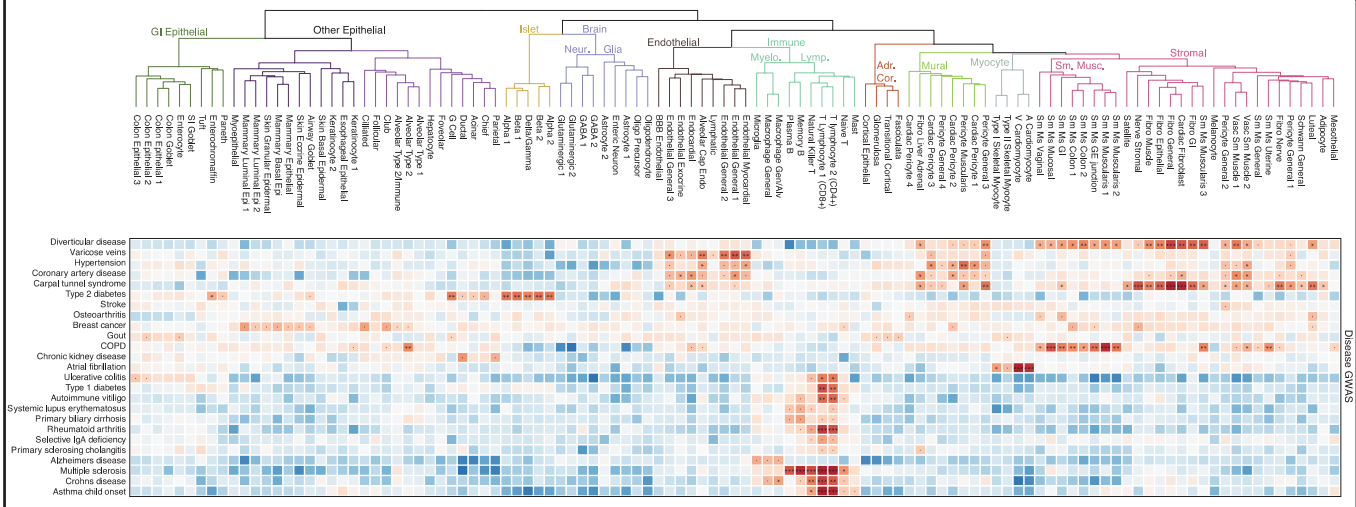
snATAC-seq in 30 Adult Human Tissues

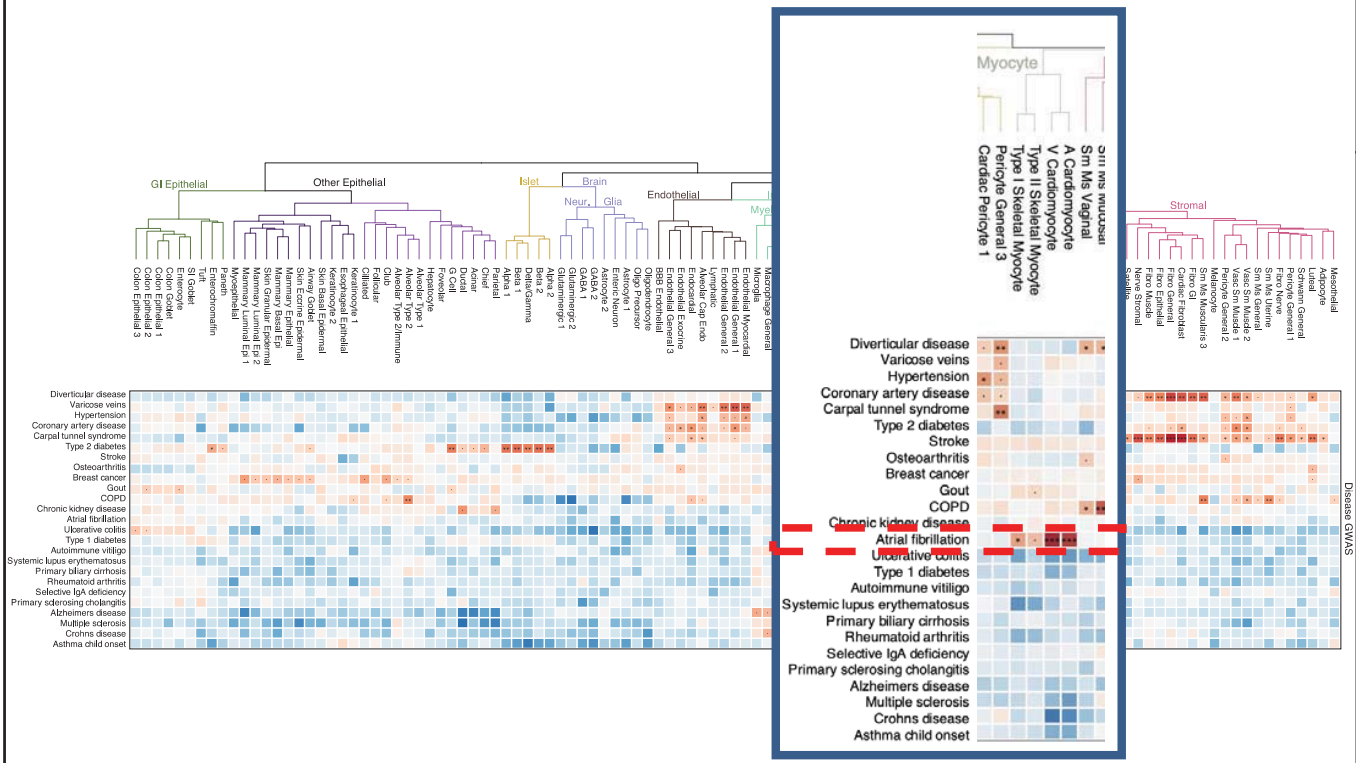




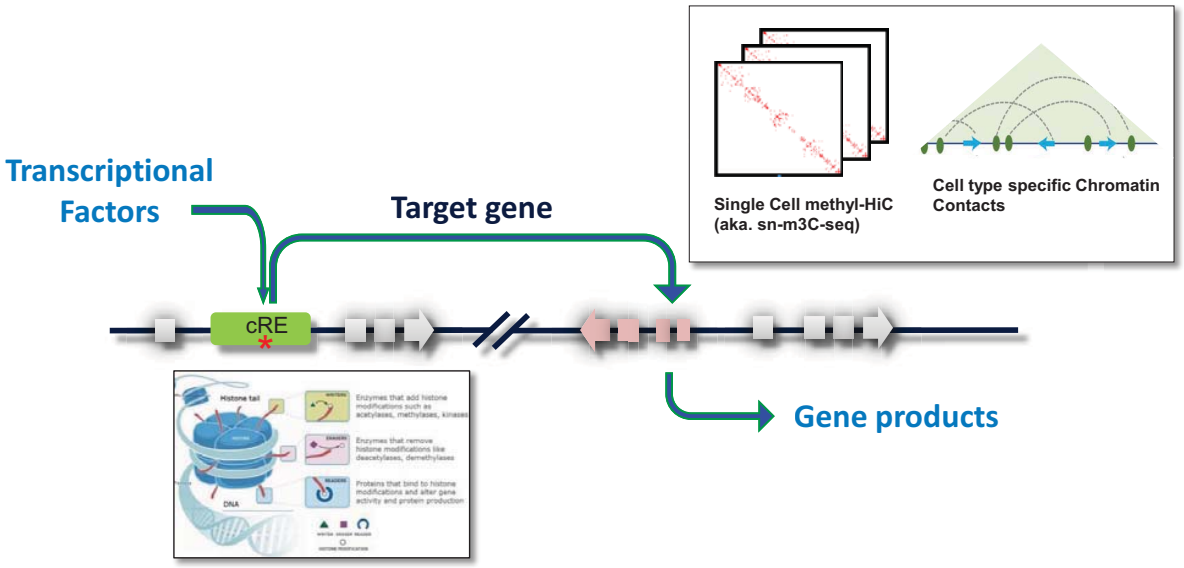
Iterative clustering

Enrichment of non-coding risk variants in candidate *cis* regulatory elements in different human cell types

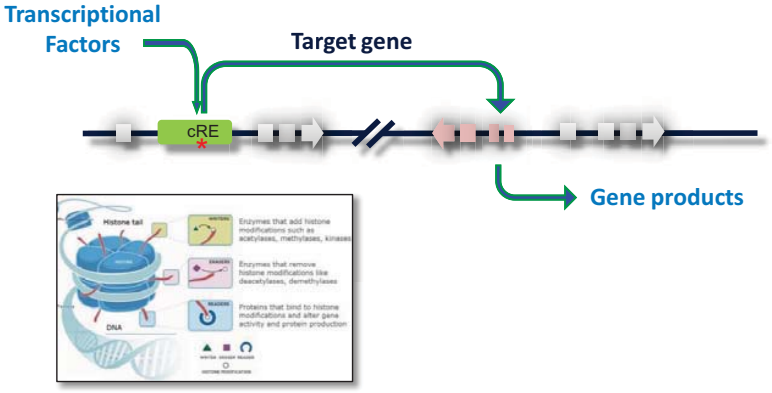


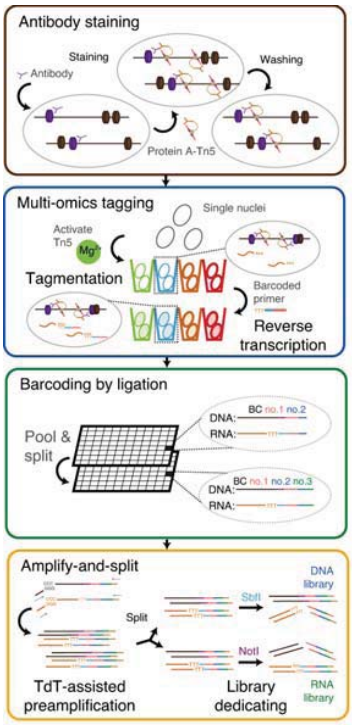


Profiling Chromatin State and the Higher-order Chromatin Structure with Single Cell Multi-omics



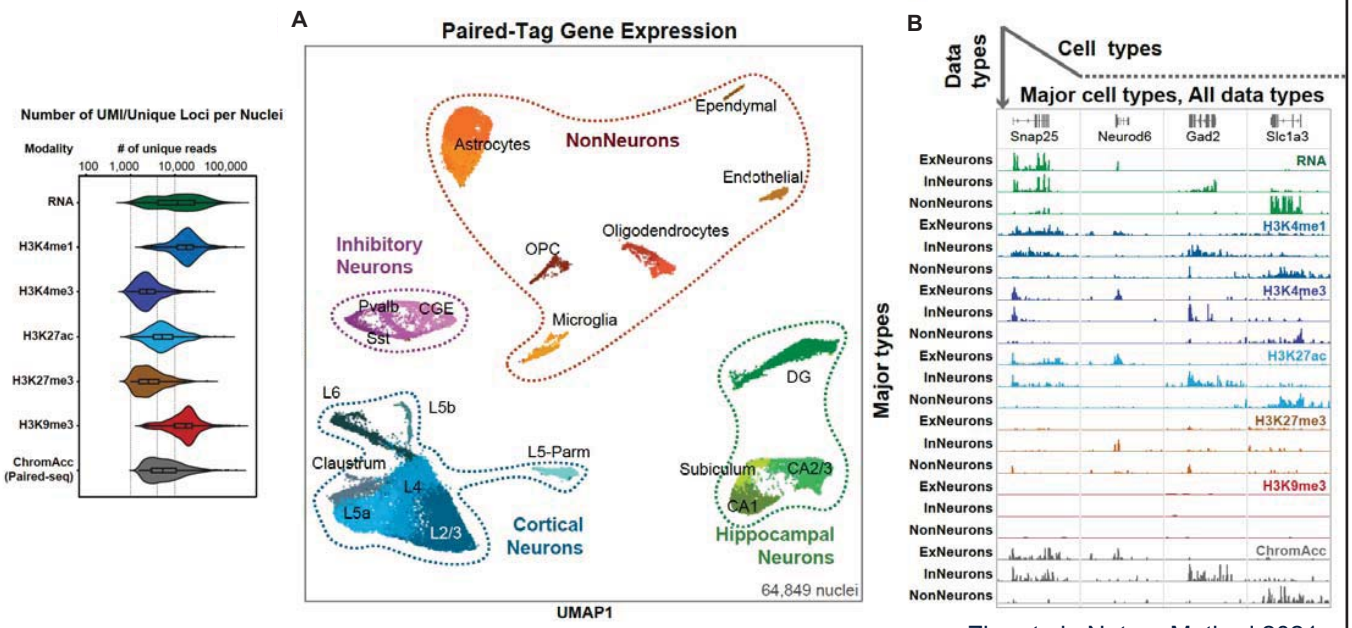
Paired-Tag: single cell co-assay of ch romatin state or TF binding and trans criptome at large scale





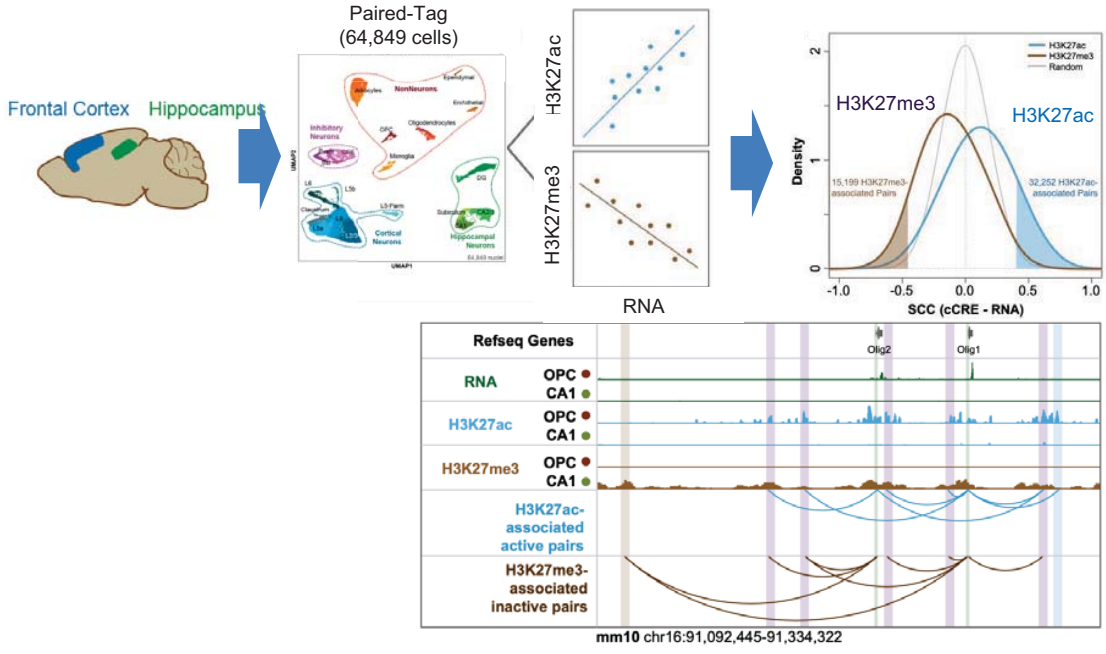
Zhu et al. Nature Method 2021

Paired-Tag analysis of adult mouse brain

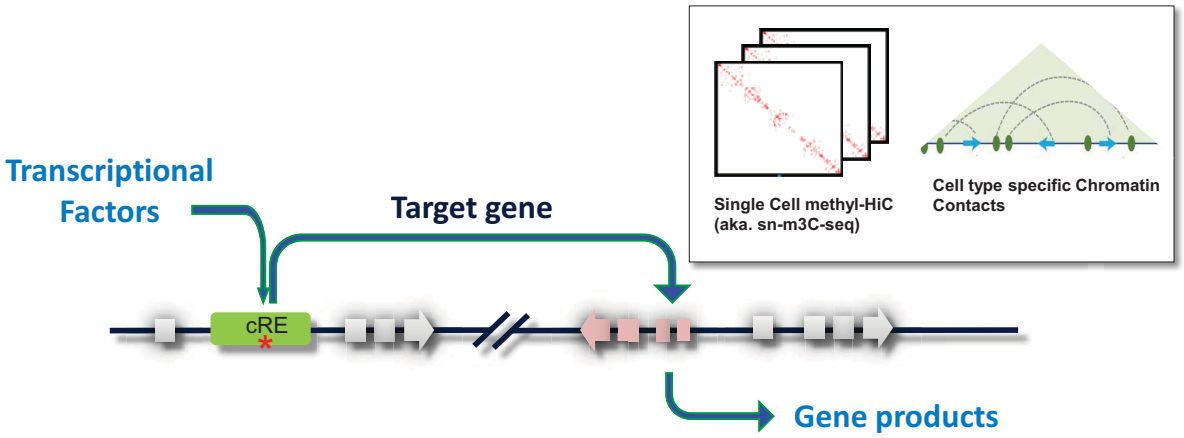


Zhu et al. Nature Method 2021

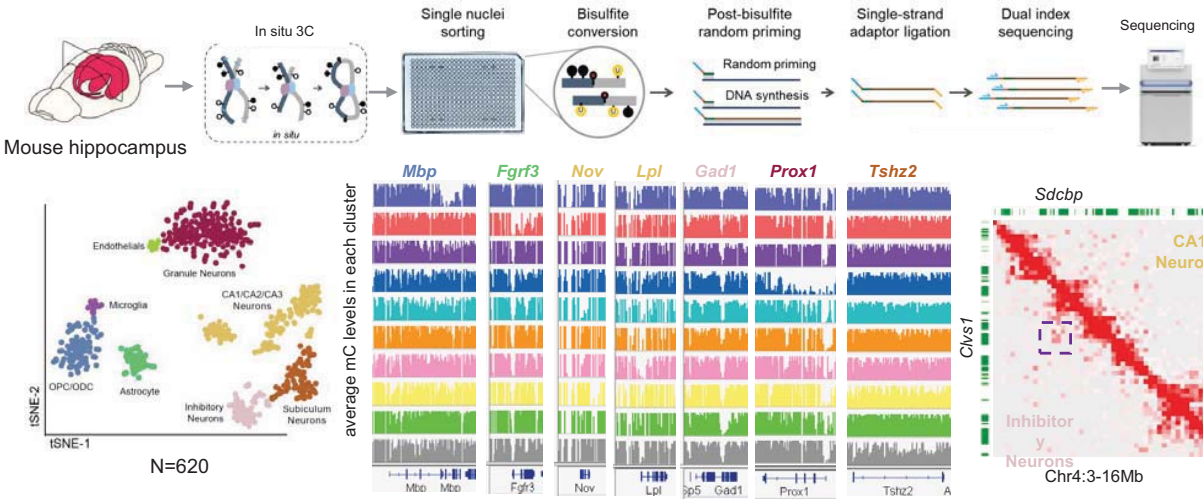
Paired-Tag analysis links distal elements to putative target genes



Profiling the Higher-order Chromatin Structure with Single Cell Multi-omics

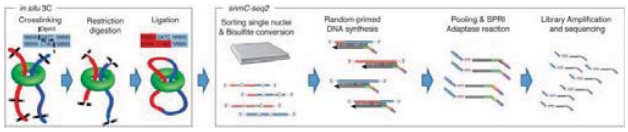


Joint Analysis of DNA Methylome and Chromatin Organization in Single Cells



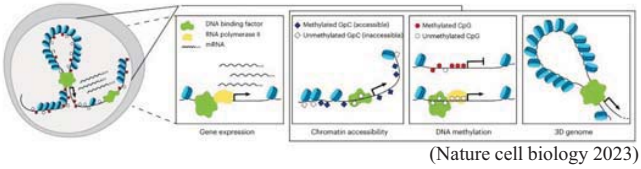
Single cell multi-omics technologies

sn-m3C-seq (3C & DNA methylation)



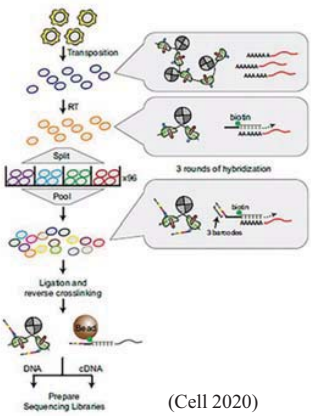
(Nature Methods 2019)

3DRAM-seq (HiC & RNA & accessibility & methylation)



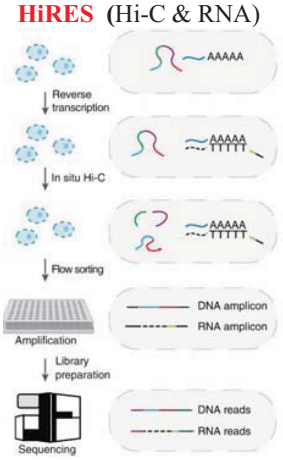
(Nature cell biology 2023)

SHARE-seq (RNA & ATAC)



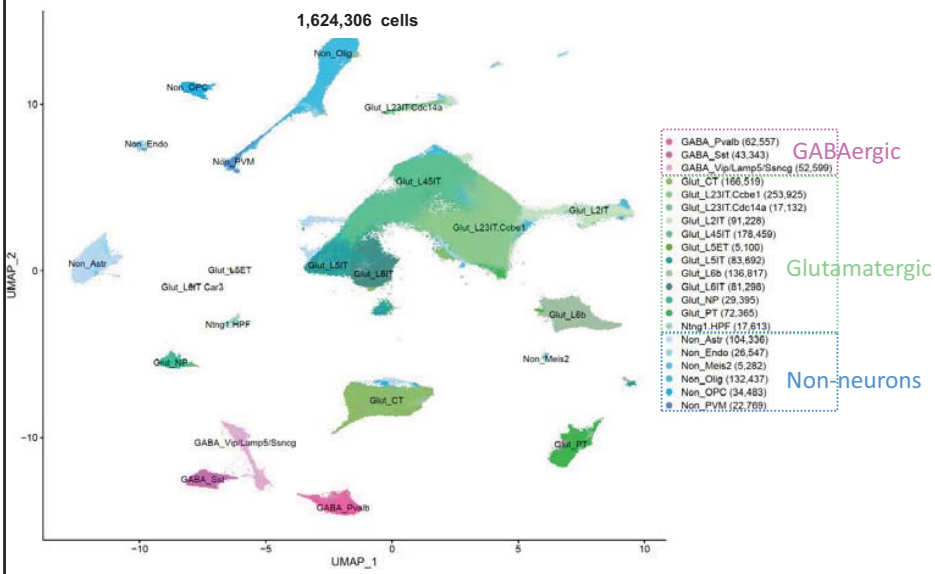
(Cell 2020)

HiRES (Hi-C & RNA)



(Science 2023)

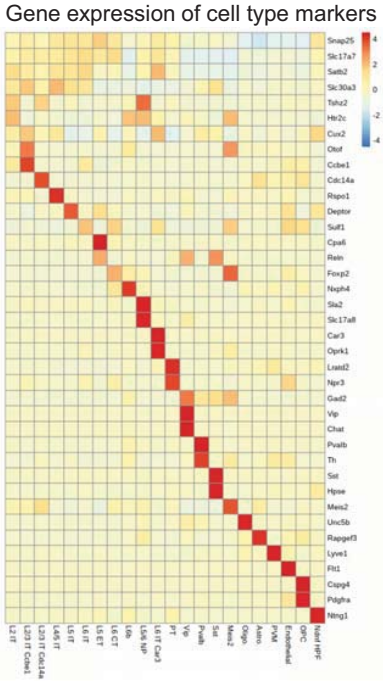
Cell annotation of 1.62 million mouse brain cells



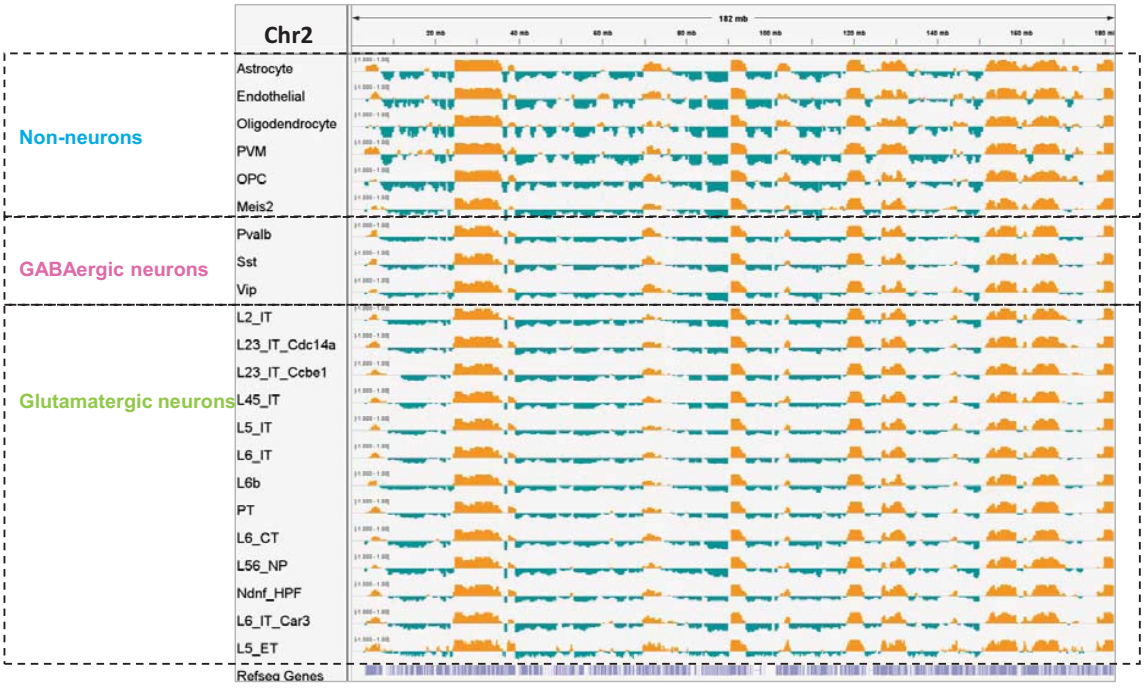
In total, 22 cell types were identified in mouse brain datasets.

average feature count per cell: 715
average UMI count per cell: 1488

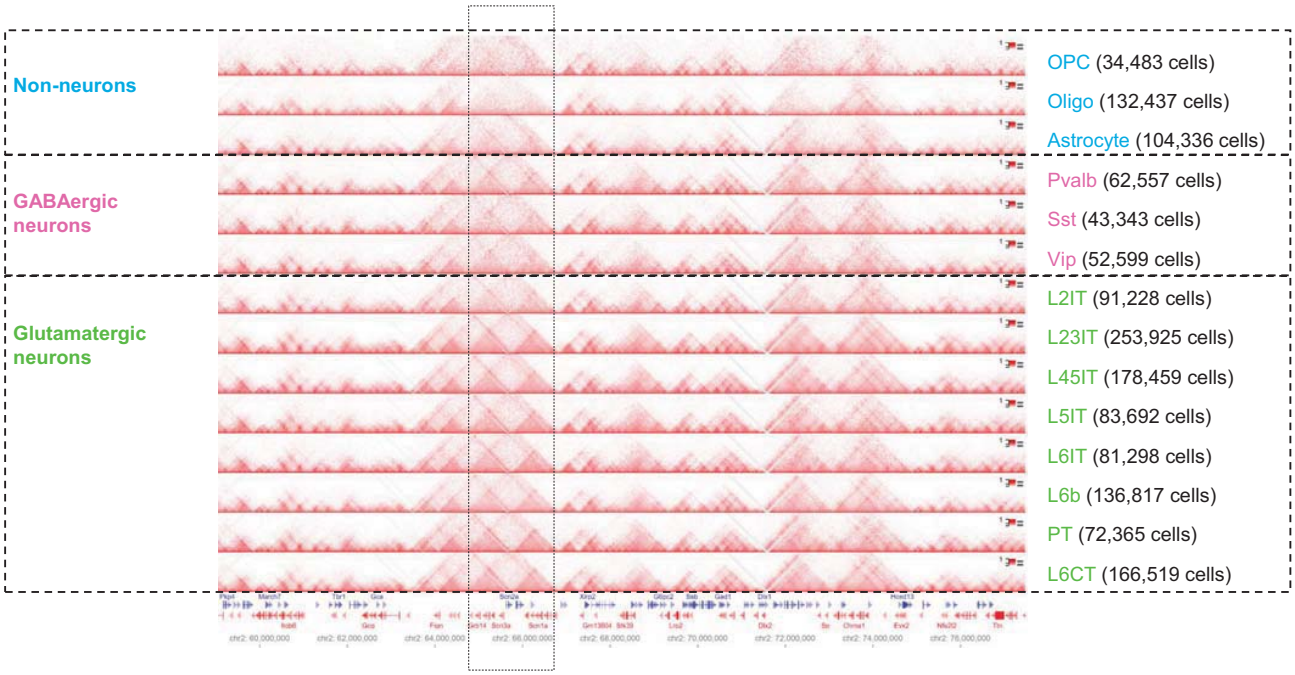
Gene expression of cell type markers



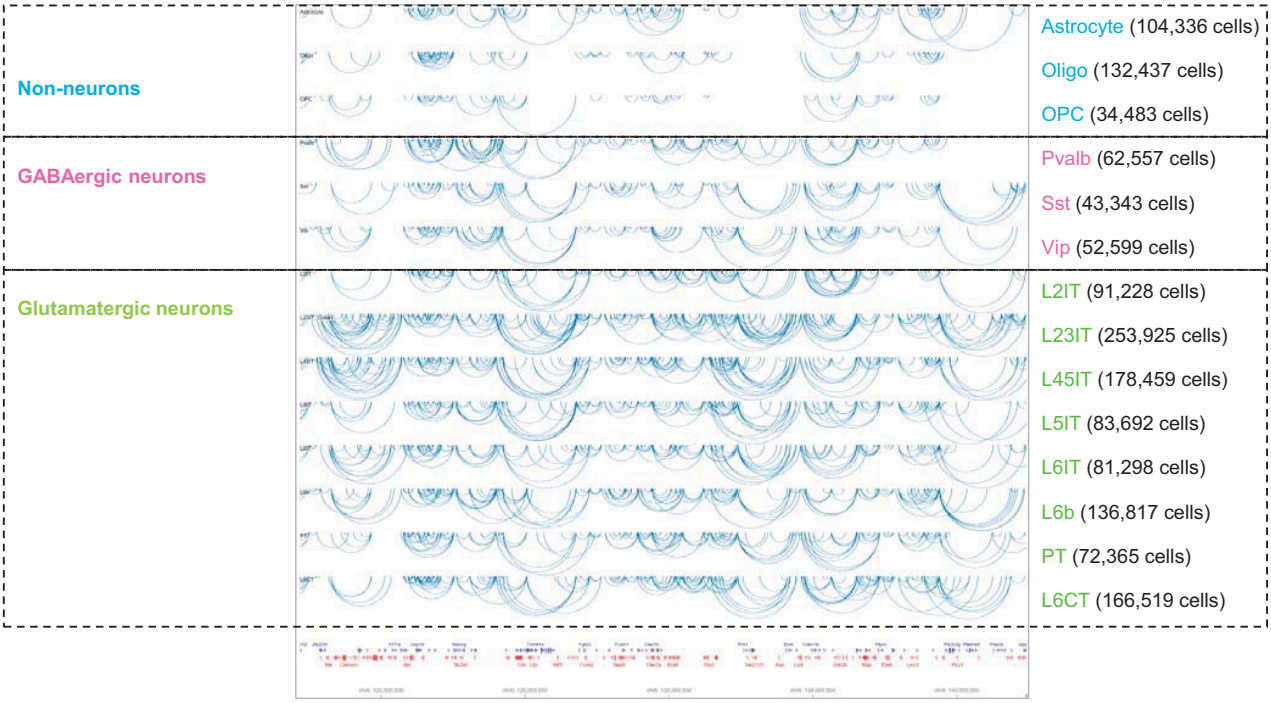
Dynamic compartment A/B patterns



Cell-type independent TAD boundary conservation



Dynamic E-P pairs across cell-types



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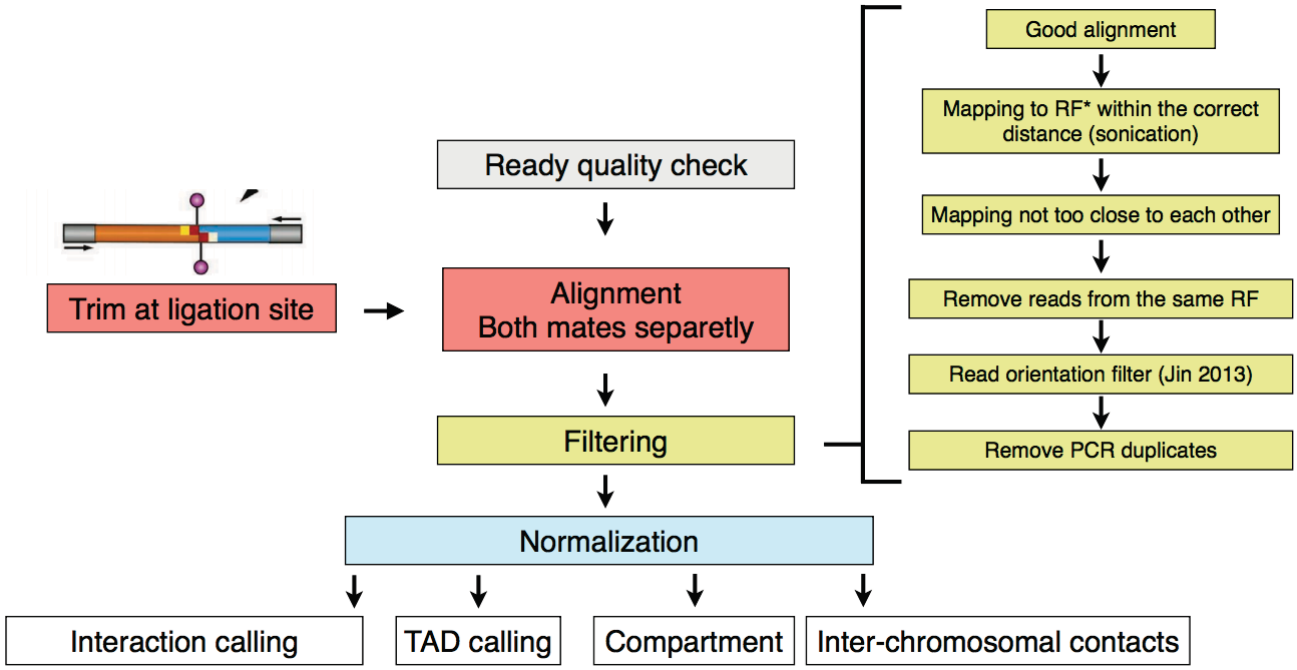
KSBi-BIML 2024

(Single-cell) 3D Epigenome Data Analysis

정인경(KAIST)

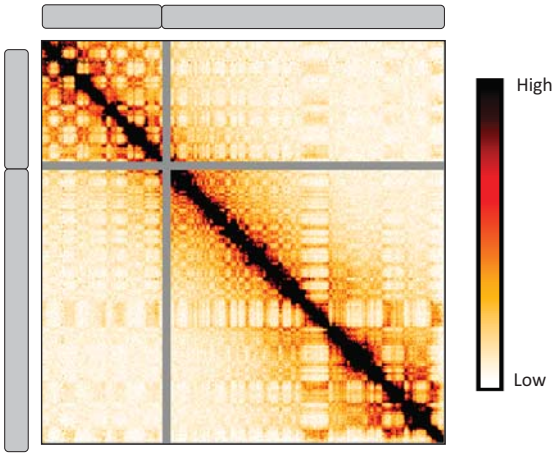
Contents

How to define interactions? - Overall Workflow



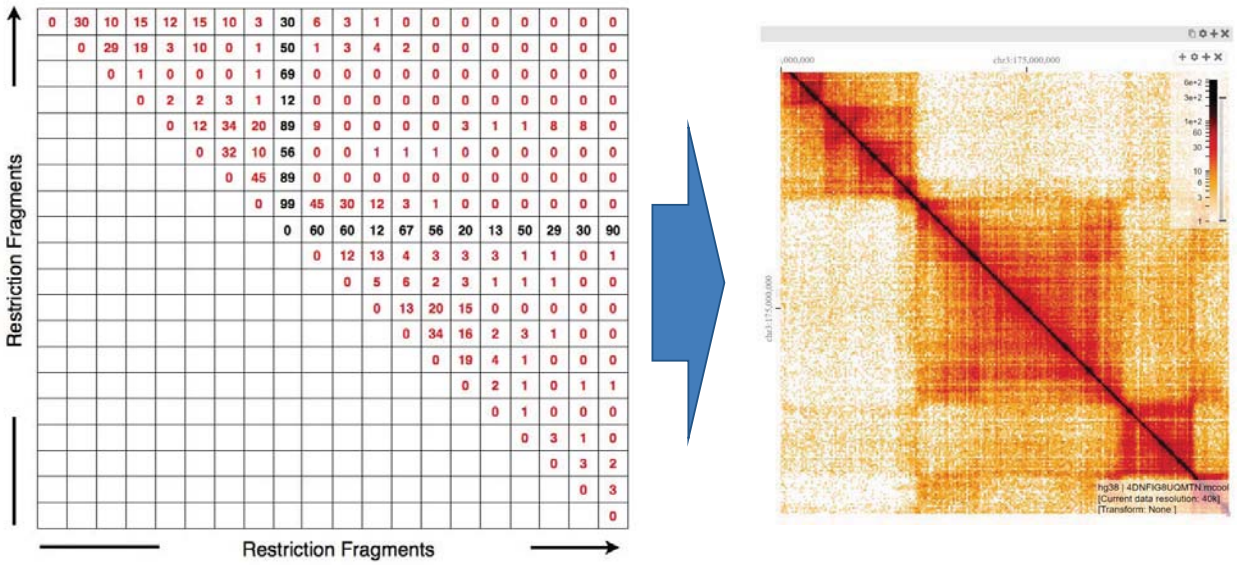
Sequencing Hi-C Library and then What?

- Put the ends back together
  - Map to a reference genome
  - Determine ligation frequency
- Binning → Matrix
  - Sizes (resolution)

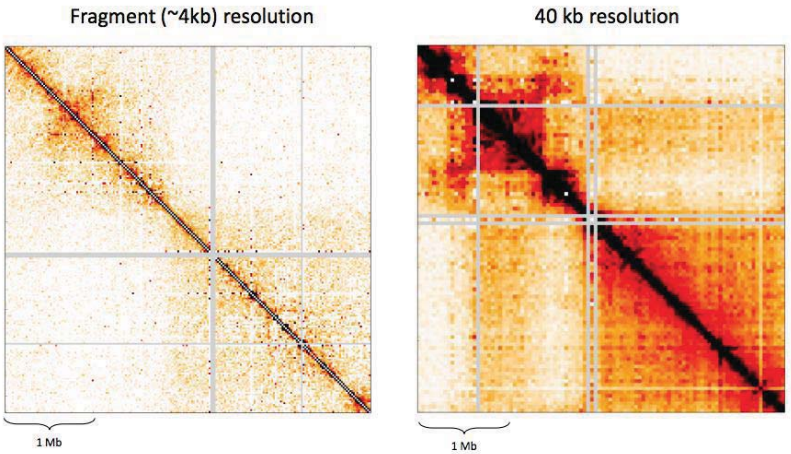


- Unless single cell, ensemble average (over 10^6 cells)
- Unless phased, averages over homologs.
- Unless synchronized, averages over the cell cycle.

Ligation Frequency Matrix (Interaction Map)



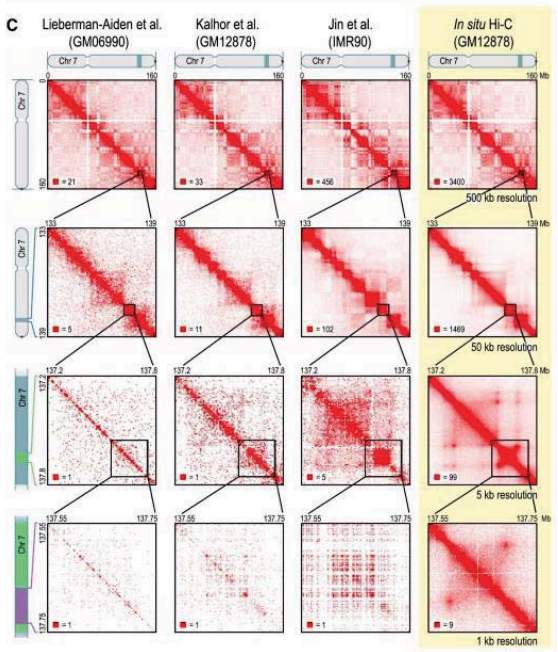
Resolution of Hi-C Interaction Map



- For the same Hi-C result, but different visualization depending on the resolution (bin size)
  - At 40kb resolution, TAD looks clear
  - At 4kb resolution location interactions look clear
- Determining optimal resolution is critical to precisely interpret Hi-C result

No clear definition to determine Hi-C resolution so far since there is no clear resolution dependent properties. However one paper proposed that **bin size can be determined as at least 80 % of all possible bins having more than 1,000 contact**

Hi-C Resolution



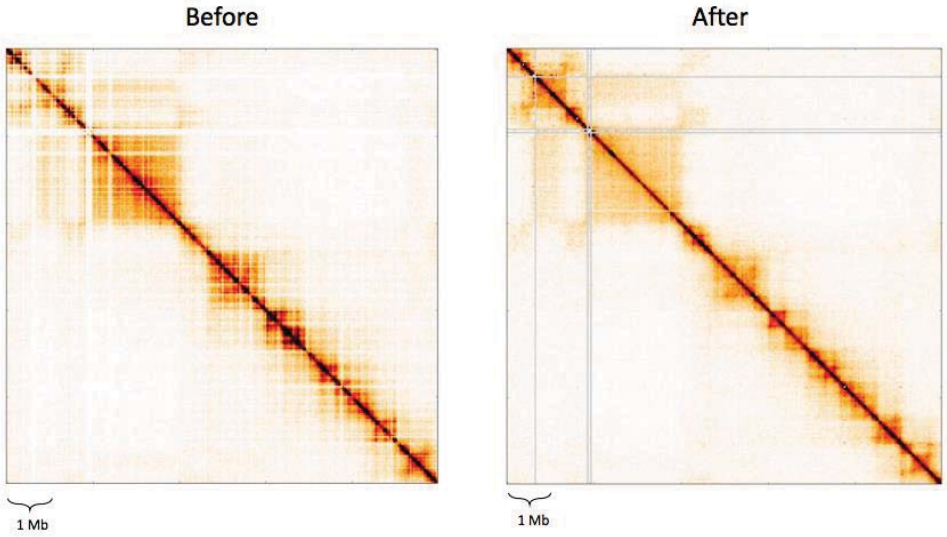
Some numbers in Hi-C resolution

- Human genome size: 3*10^9bp
- Restriction fragment length: ~4kb (6bp cutter)
- Number of fragments: 7.5*10^5
- Total interaction space: 5.6*10^11
- Number of cells per experiment: 10^6
- When we have 200M usable reads (2*10^8), 200 interactions were measured per cell
- Interaction space is under-sampled

How can we increase Hi-C resolution?

- Reduced interaction space (targeted approaches)
- Increased sequencing depth
- Reduced fragment size

Bias Correction (Normalization)



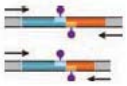
Uncorrected

Corrected (balanced)

Two major approaches for bias correction

Digestion efficiency as a function of sequence composition (and DNA compaction)

Ligation efficiency as a function of fragment length



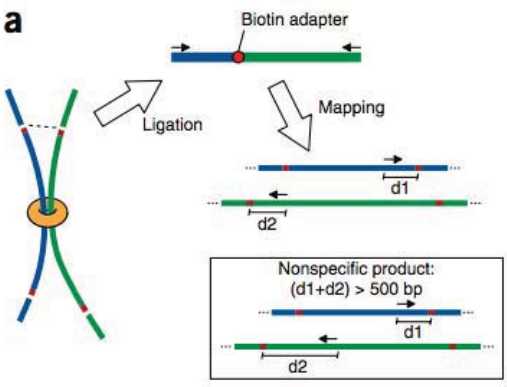
Sequencing efficiency as a function of sequence composition

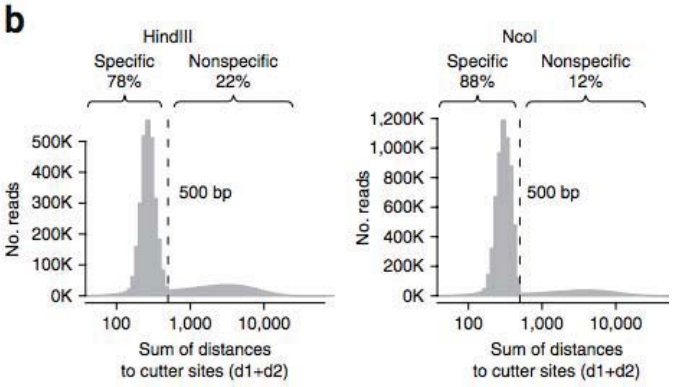
1. Explicit factor methods (ex: HiCNorm)
   - Model bias due to GC content, fragment lengths, etc.
2. Coverage based methods (ex: ICE)
   - Don't model explicit sources of bias. Only assumes factorizable biases

Bias correction with explicit factor methods

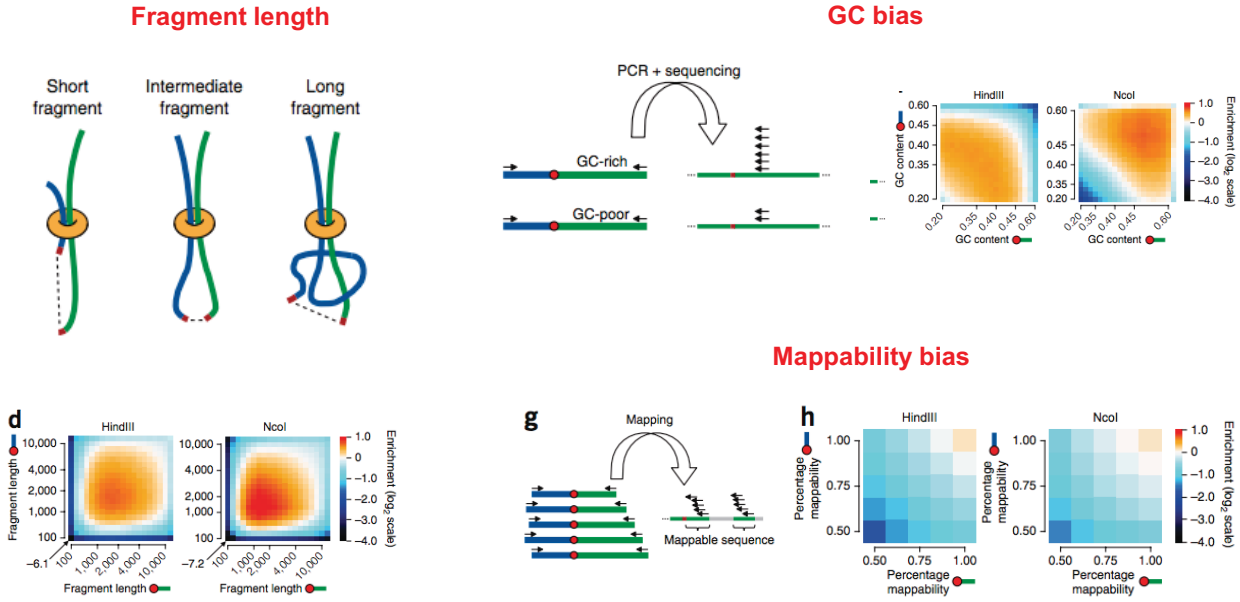
- Yaffe and Tanay or HiCNorm used explicit bias model based on 3 features that cause biases in Hi-C result
  - GC content, mappability, and effective length (or fragment length).
  - External information dependent
  - Effect of GC content on Hi-C library is enzyme-dependent.

Filtering for random ligation events





Bias correction with explicit factor methods



Bias correction with explicit factor methods

Let $\boldsymbol{U^i} = \{u^i_{jk}\}_{1\le j,k\le n_i}$ represent the $n_i \times n_i$ Hi-C *cis* contact map for chromosome i, where n_i is the number of consecutive, disjoint 1 MB bins in chromosome i. Each entry u^i_{jk} represent the number of paired-end reads spanning two bins L^i_j and L^i_k. Let x^i_j, y^i_j and z^i_j represent the effective length feature, the GC content feature and the mappability feature at locus j for chromosome i, respectively. Similarly, let x^i_k, y^i_k and z^i_k represent the effective length feature, the GC content feature and the mappability feature at locus k for chromosome i, respectively. We assume that u^i_{jk} follows Poisson distribution with rate θ^i_{jk}:

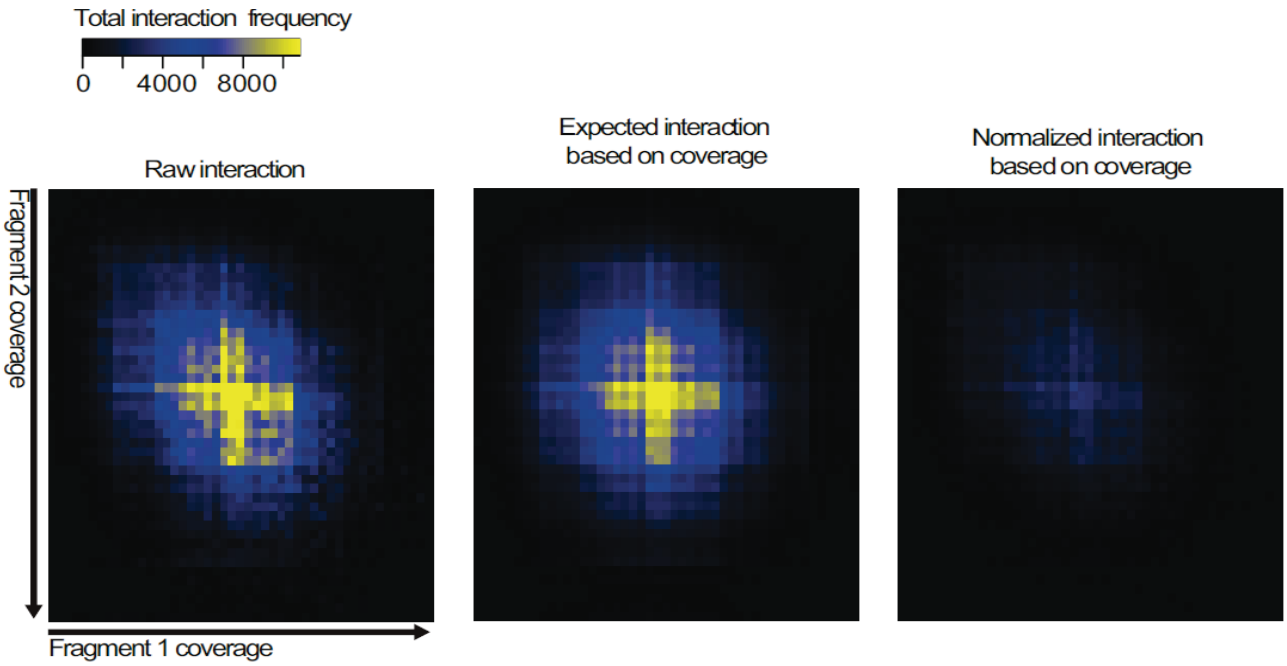
$$\log(\theta^i_{jk}) = \beta^i_0 + \beta^i_{len}\log(x^i_j x^i_k) + \beta^i_{gcc}\log(y^i_j y^i_k) + \log(z^i_j z^i_k).$$

Here β^i_0 is the intercept term. β^i_{len} and β^i_{gcc} represent the effective length bias and the GC content bias, respectively. $\log(z^i_j z^i_k)$ is the Poisson offset term of the mappability bias. We fit this Poisson regression model, and let $\hat{\beta}^i_0$, $\hat{\beta}^i_{len}$ and $\hat{\beta}^i_{gcc}$ represent the corresponding parameter estimates. We further define the estimated Poisson rate $\hat{\theta}^i_{jk}$ as following:

$$\hat{\theta}^i_{jk} = exp\{\hat{\beta}^i_0 + \hat{\beta}^i_{len}\log(x^i_j x^i_k) + \hat{\beta}^i_{gcc}\log(y^i_j y^i_k) + \log(z^i_j z^i_k)\}.$$

The residual $e^i_{jk} = u^i_{jk}/\hat{\theta}^i_{jk}$ is the normalized *cis* interaction between two bins L^i_j and L^i_k.

HiCNorm / covNorm



Bias correction with coverage based methods

- Do not try to identify sources of biases but learn their effect from data

$$f_{ij} = \frac{c_{ij}\left(\sum_{k=1}^{K-1}\sum_{l=k+1}^{K} c_{kl}\right)}{\left(\sum_{k=1}^{K} c_{ik}\right)\left(\sum_{k=1}^{K} c_{kj}\right)}$$

f_{ij}: normalized ligation frequency between segments i and j

c_{ij}: number of reads between segments i and j

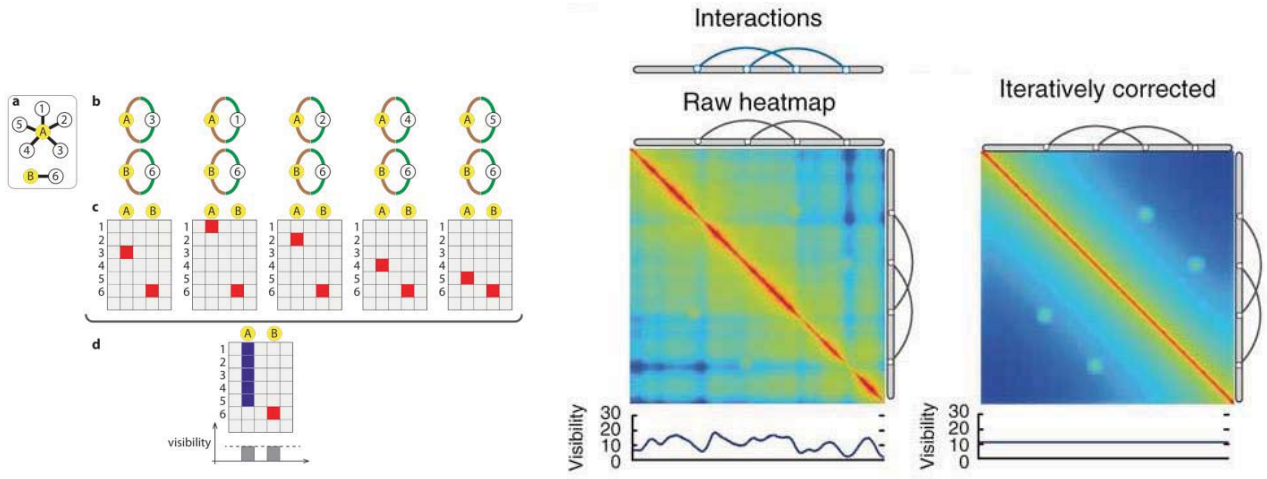
$\sum_{k=1}^{K-1}\sum_{l=k+1}^{K} c_{kl}$: Total number of reads

$\sum_{k=1}^{K} c_{ik}$: Total number of for segment i

$\sum_{k=1}^{K} c_{kj}$: Total number of for segment j

Bias correction with coverage based methods - equalized visibility

ICE equalized visibility: Each bin has equal coverage



Imakaev *et al* (2012)

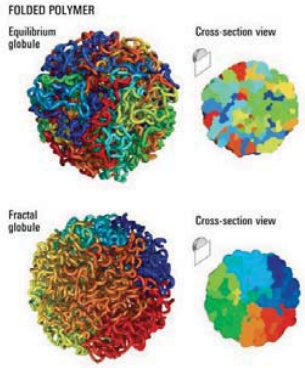
Bias correction with coverage based methods - equalized visibility

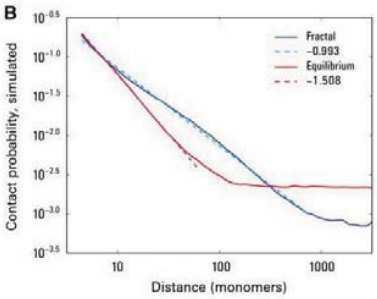
Observed $O_{ij} = B_i B_j T_{ij}$ True (Biases)

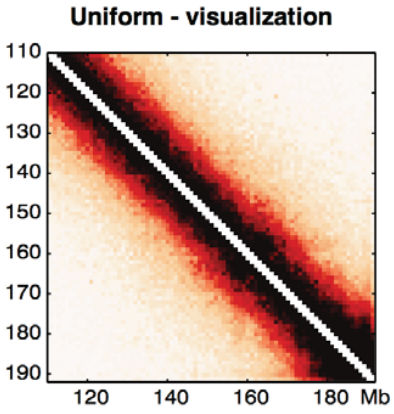
1. Start from Wij (=Oij) as the iterative process gradually changes this matrix to Tij
2. Calculate coverage of i as s_i=sum_j(Wij)
3. Additional biases delta Bi are calculated by renomralizitng s_i to have the unit mean as delta Bi = S_i / mean(S_i)
4. New Wij = Wij / (delta Bi * delta Bj)
5. Iterate step 2-4 until the variance of the additional biases becomes negligible

Issues:
- local signals can be removed
- interaction hubs such as transcriptional factory are not covered by ICE normalization

Random collision (Distance Dependent Ligation Events)



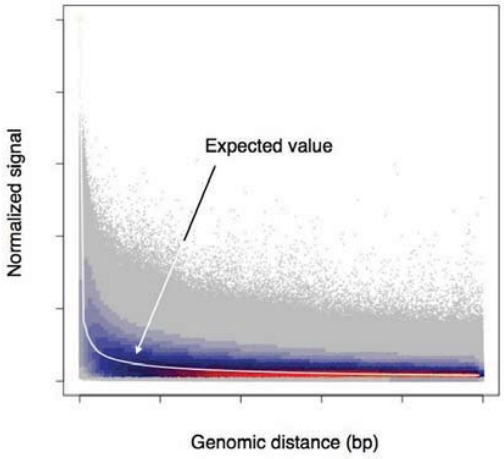


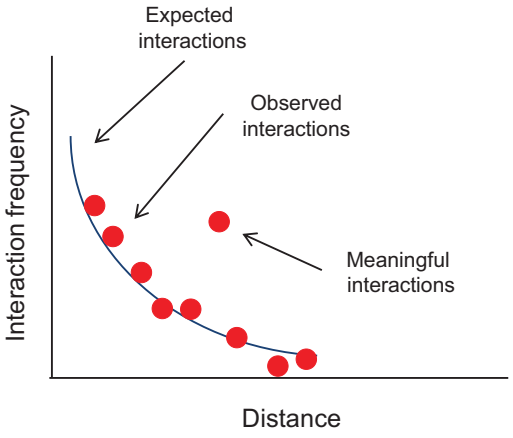


Random collision events

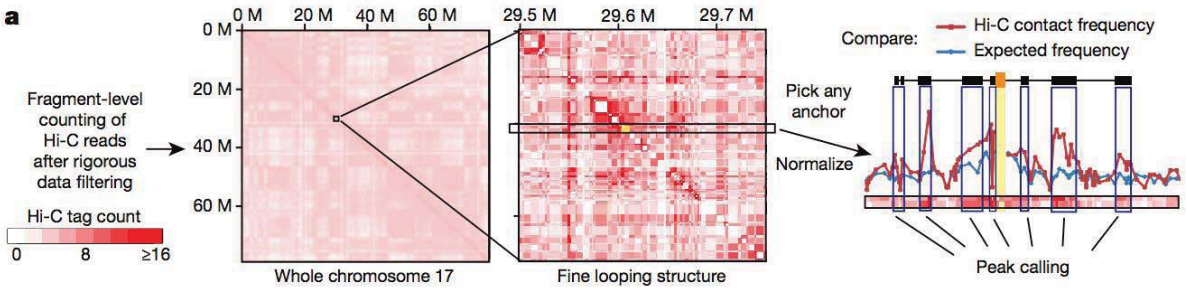
- Interaction occurring just because chromatin is a biopolymer and folds
- Random collisions can be estimated as expected interaction strength at a particular distance

Random collision (Distance Dependent Ligation Events)



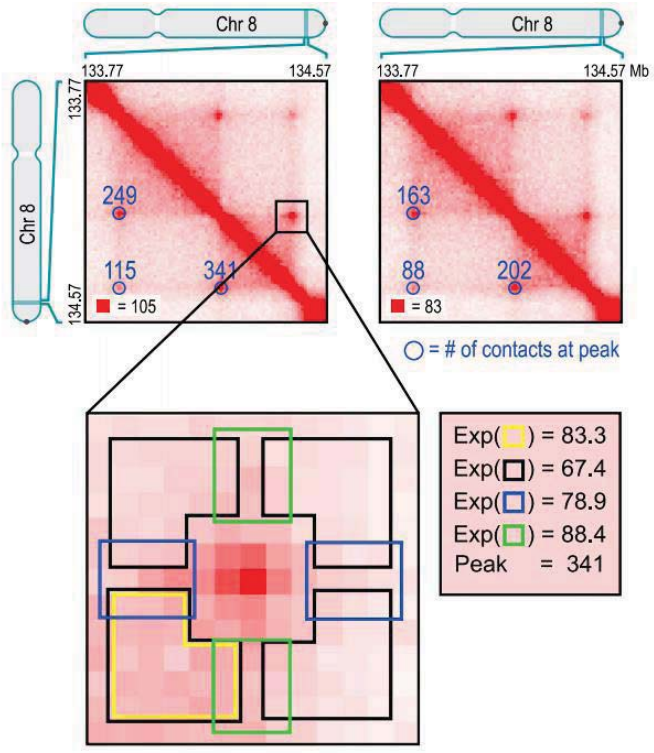


Detection of biologically meaningful interactions

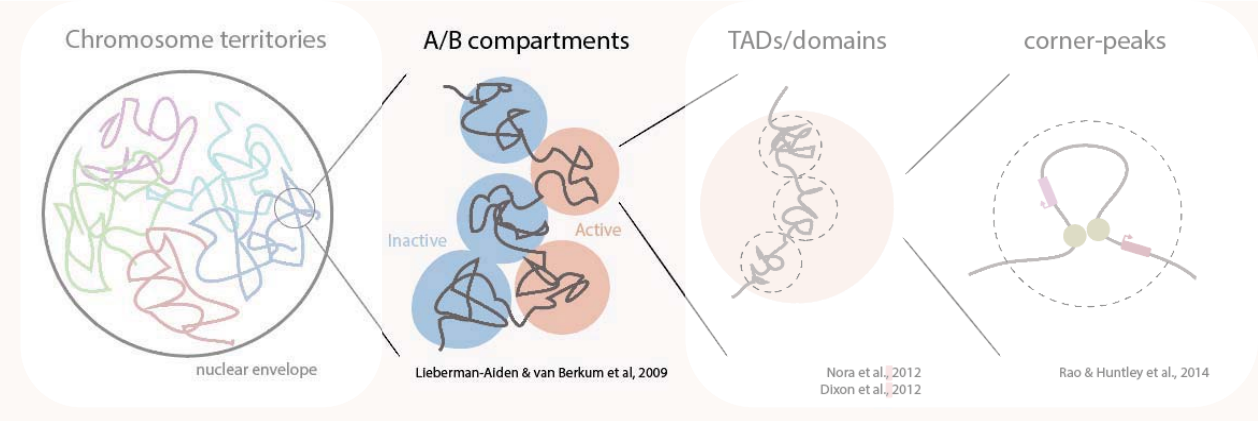


- Apply negative binomial distribution
- Test whether its strength is unexpectedly high given the biases, distances, and additional signal strength threshold

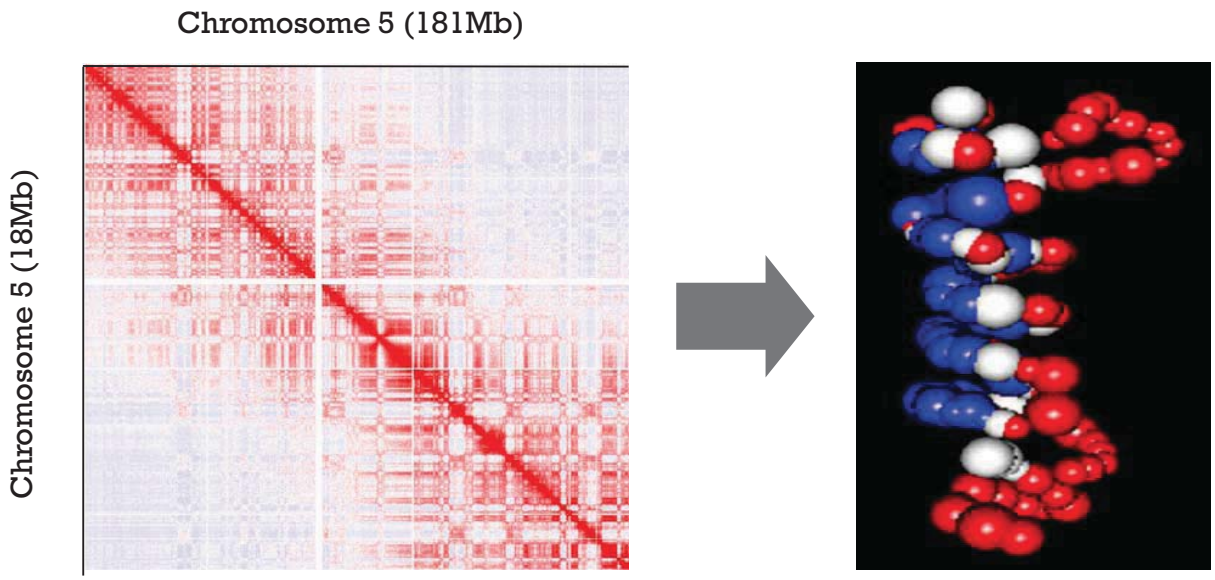
Detection of biologically meaningful interactions



- HiCCUPS
- Considering local background
- Pixels in the middle should have signal 50% higher than the surrounding



Modeling 3D chromatin structure from Hi-C contact map

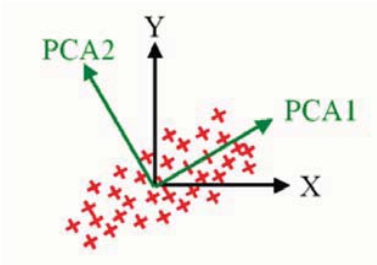


Hi-C Contact Map

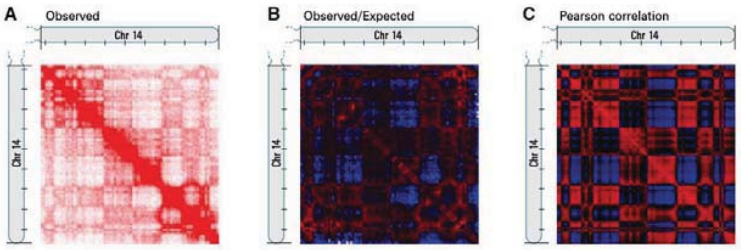
3D modelling

PCA (Principle Component Analysis) to contact matrix

Find component axes that maximize variance
(use the first eigenvector *v* of the PCA/SVD)



Apply PCA

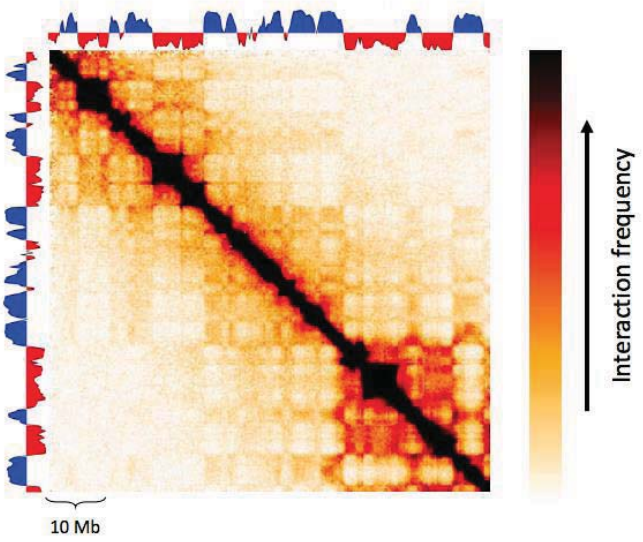


v is the vector that minimizes $||svv^T-A||$, thus Aij will be near svivj

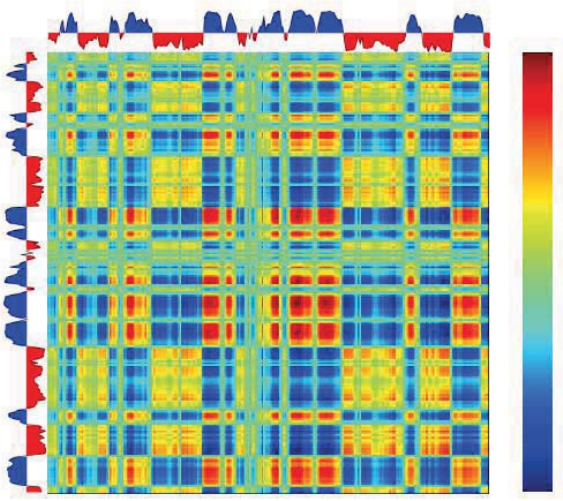
So, if vi and vj have the same sign their product will be positive (higher interaction bins i and j)

if vi and vj have the opposite sign their product will be negative (low interaction bins i and j)

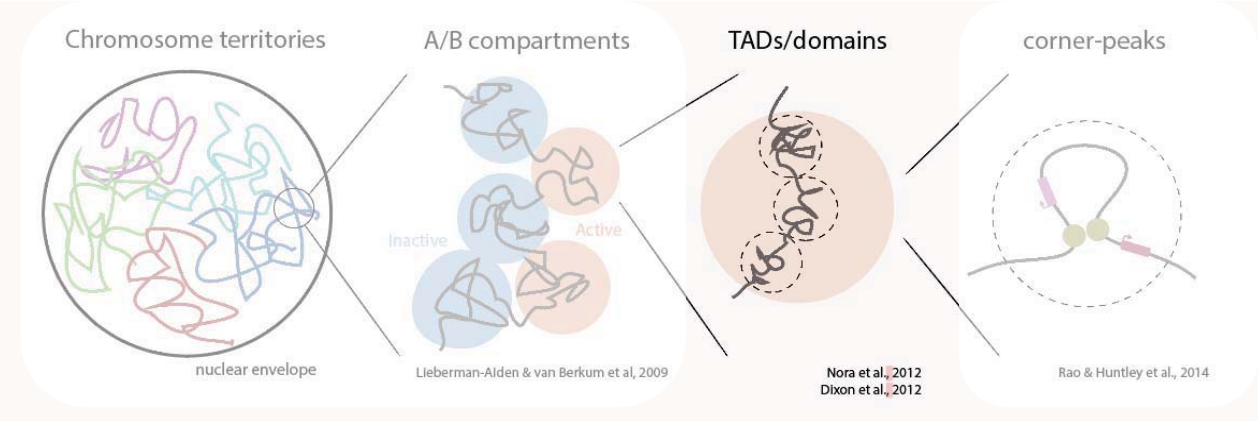
Apply PCA to 3D chromatin structure to find a major structural component



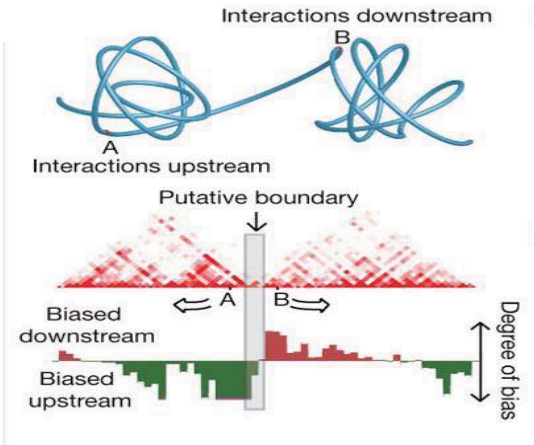
Interaction frequency matrix



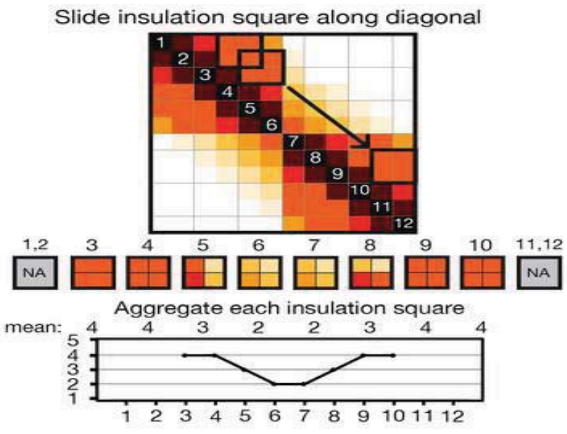
cor(Interaction frequency matrix)



Methods to define TAD boundaries



Directional Index Scores

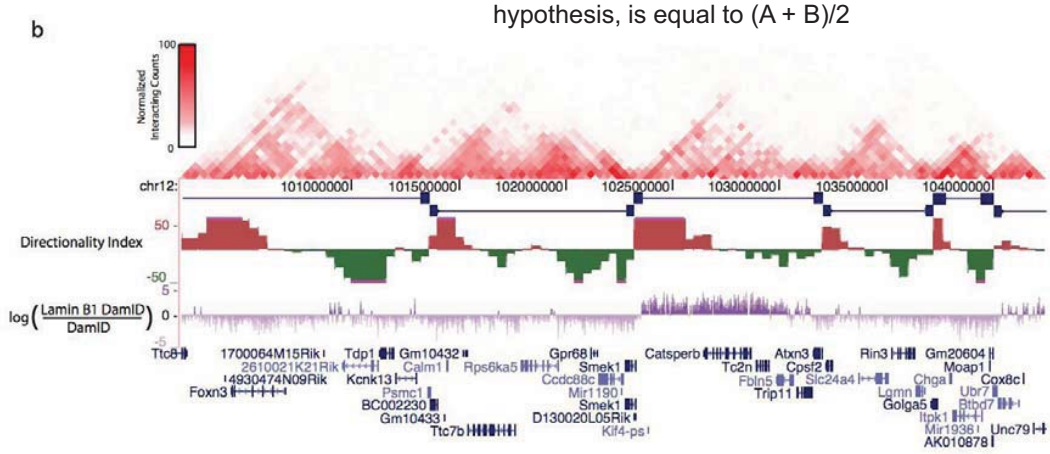


Insulation Scores

Methods to define TAD boundaries – Directionality

$$DI = \left(\frac{B - A}{|B - A|}\right)\left(\frac{(A - E)^2}{E} + \frac{(B - E)^2}{E}\right)$$

A: the number of reads that map from a given locus to the upstream 2Mb
B: the number of reads that map from the same locus to the downstream 2Mb
E: the expected number of reads under the null hypothesis, is equal to (A + B)/2



Dixon *et al*., (2012)

KSBI | 한국생명정보학회

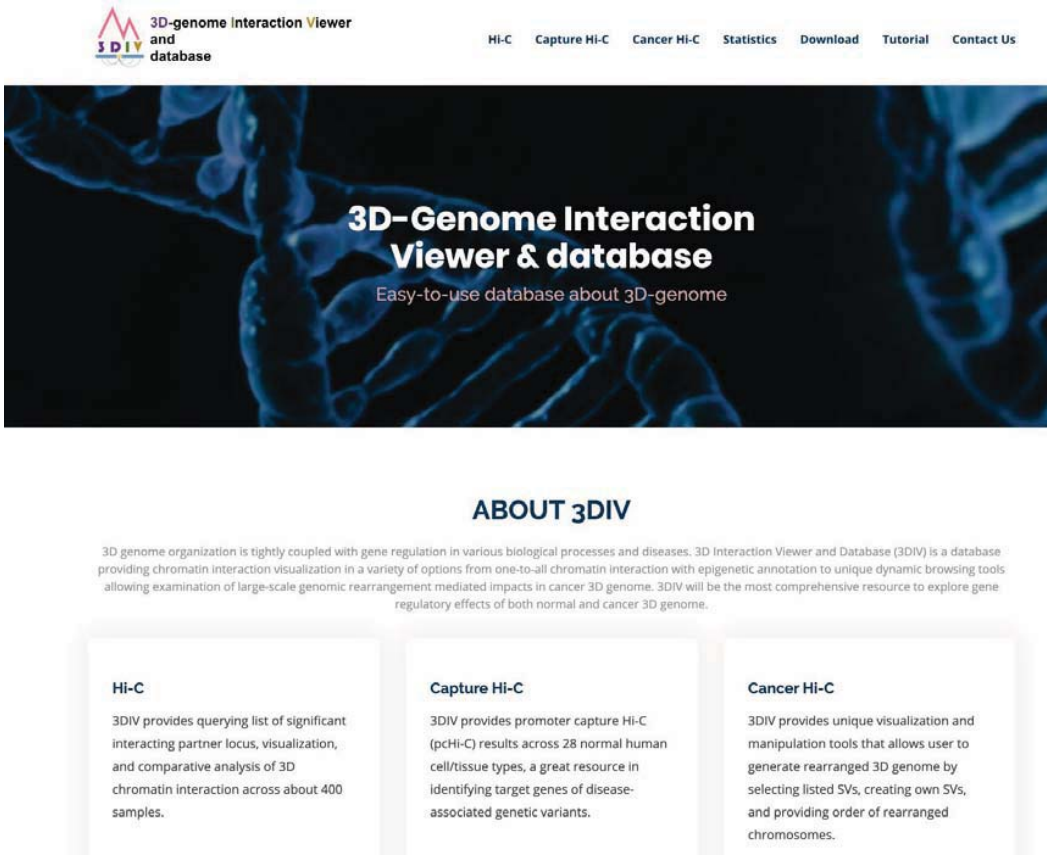
KSBi-BIML 2024

(Single-cell) 3D Epigenome Data Analysis

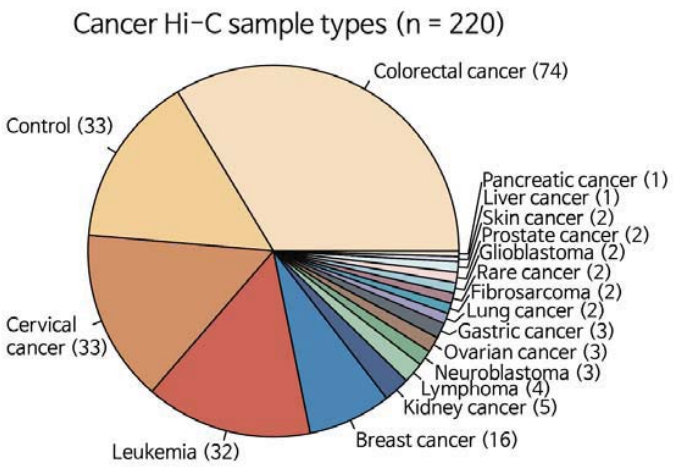
정인경(KAIST)

Contents

3DIV: http://3div.kr



Hi-C data collection in 3DIV



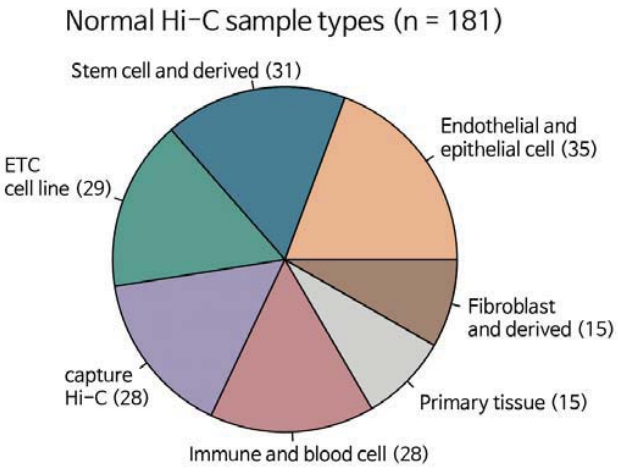


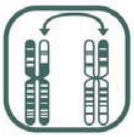
Table 1. Comparison of the updated 3DIV and other 3D genome databases as of October 2020

Software	Number of samples[a]	Hi-C contact map	TAD annotation	One-to-all interaction	Interaction table	Distance normalization	Interactive Hi-C contact map browsing	Live manipulation of genomic rearrangement	Structural variation annotation
3DIV 2021 Update	401	✓	✓	✓	✓	✓	✓	✓	✓
3DIV	80	✓	✓	✓	✓	✓	✓		
4D Nucleome	337[b]	✓					✓		
Nucleome Browser	138[c]	✓					✓		
WashU Epigenome Browser	36[c,d]	✓					✓		
HiView	2		✓	✓	✓	✓	✓		
HUGIn2	83	✓	✓	✓	✓	✓			
3D Genome Browser	113	✓	✓						
GITAR	20[c]	✓	✓						
Hi-C Data Browser	69	✓		✓					

Unique functionalities of 3DIV

Features of 3DIV

187 cancer/tumor tissue samples with 33 control samples



Pan-cancer SV data for corresponding cancer type

153 cell line/tissue Hi-C and 28 promoter capture Hi-C data

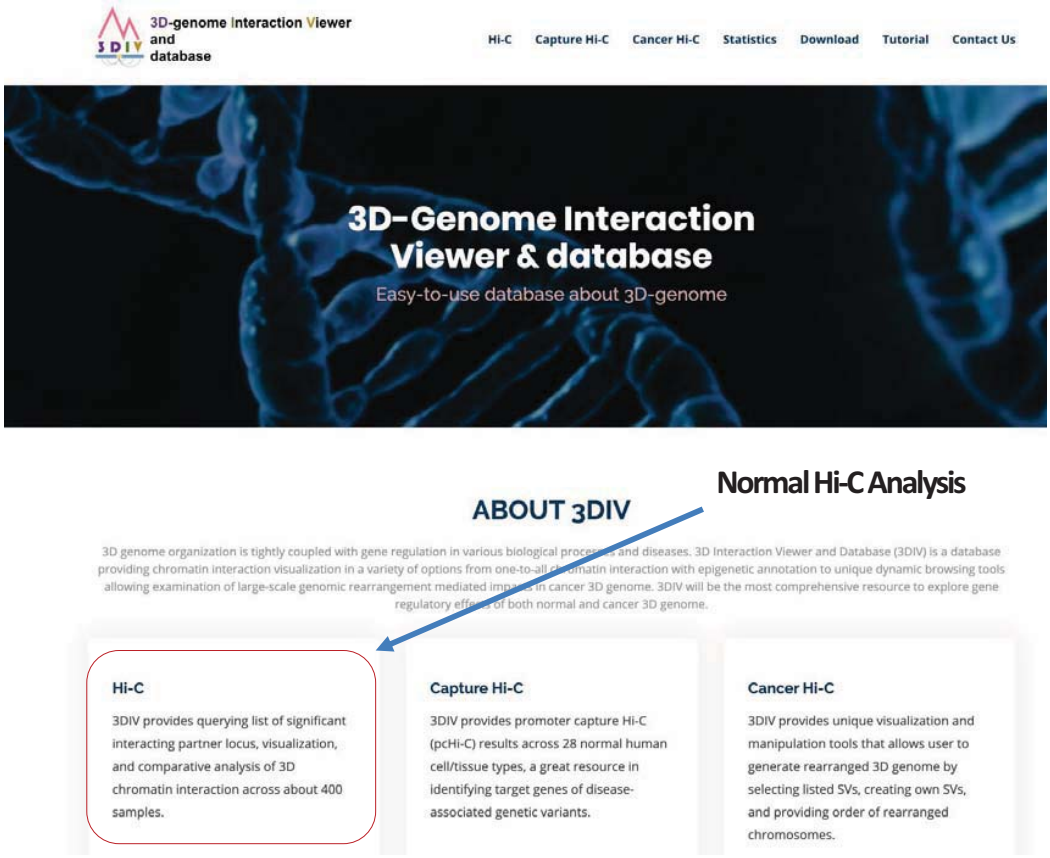
MySQL + Java Spring + HTML5 based webserver implementation

230 billion reads processed and normalized Hi-C contact maps

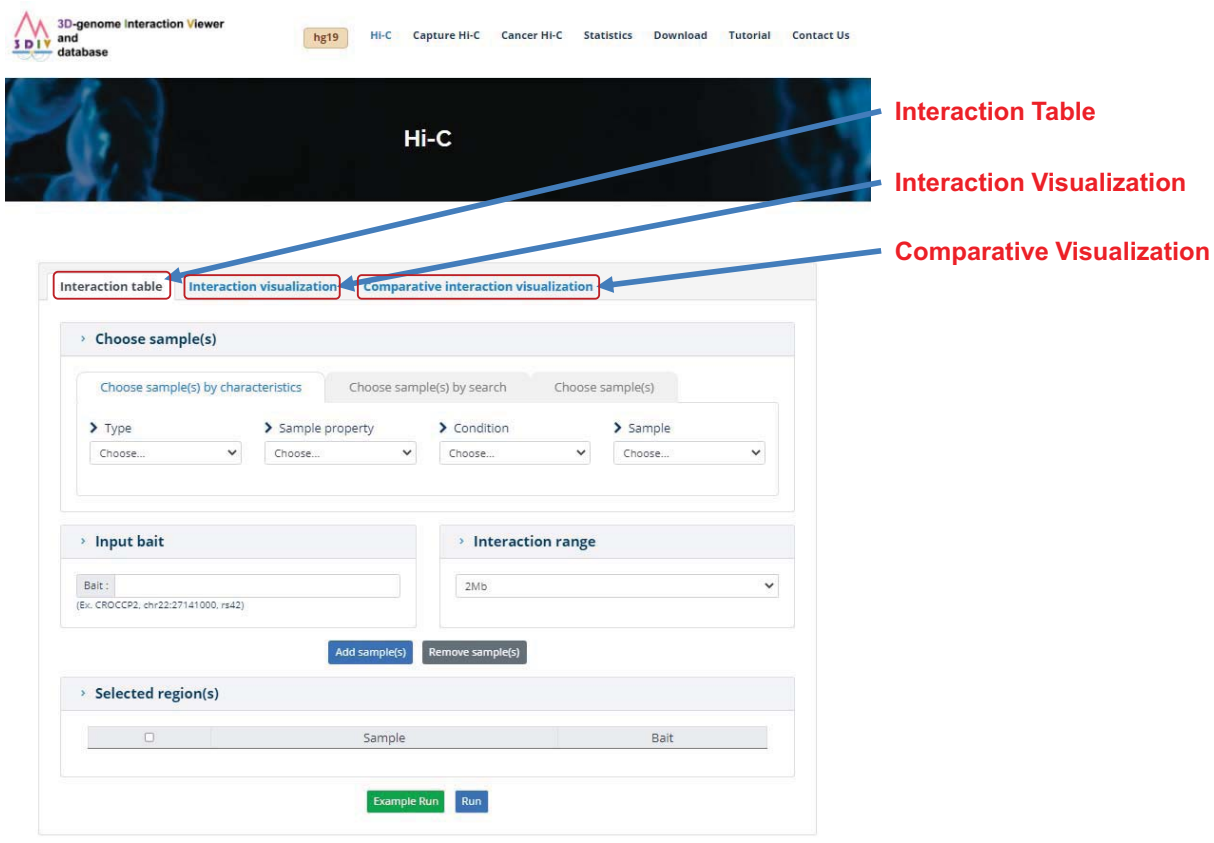
Interactive visualization function on web page

Normal Hi-C Analysis

Normal Hi-C Analysis



Normal Hi-C Analysis



Functionalities of normal Hi-C in 3DIV

Interaction Table

- Bias-removed/distance-normalized Interaction frequency
- Disease-associated GWAS SNPs
- Promoter/Enhancer/super-enhancer annotation
- Histone ChIP-seq signal

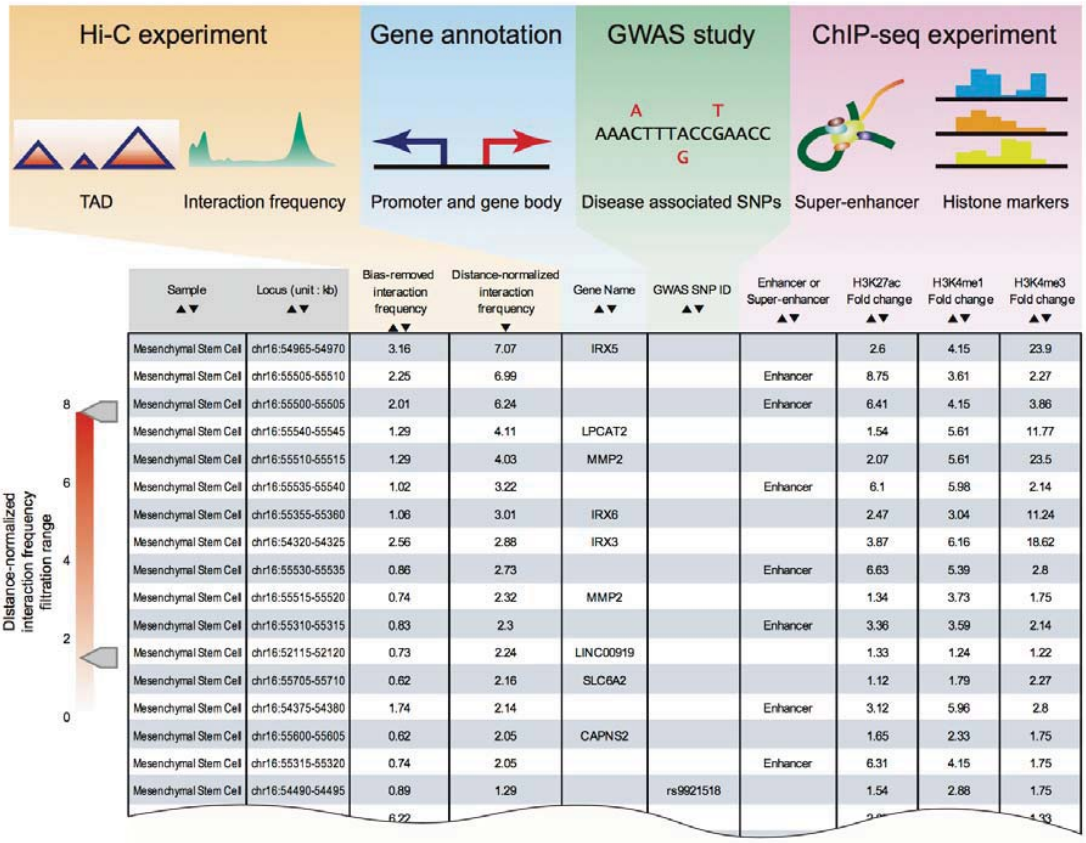
Interaction Visualization

- Interaction frequency heatmap
- Topologically associating domains
- One-to-all interaction plot
- Arc-representation of significant interactions

Comparative Visualization

- Comparative interaction frequency heatmap
- Synchronized interaction visualization

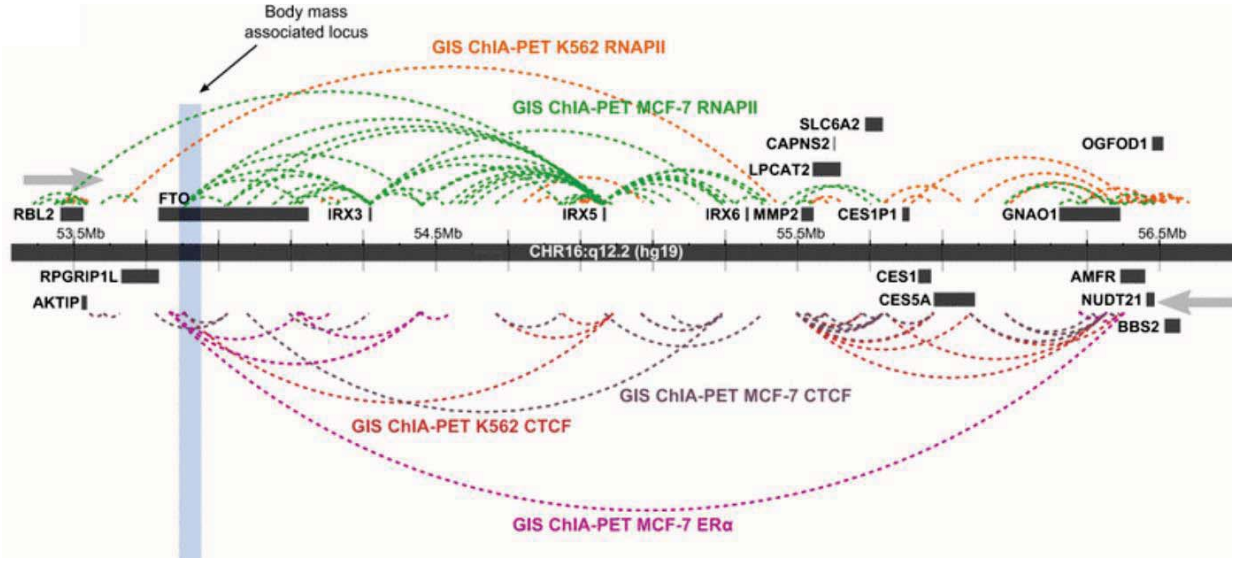
Module 1 : Interaction Table



Sample	Locus (unit : kb)	Bias-removed interaction frequency	Distance-normalized interaction frequency	Gene Name	GWAS SNP ID	Enhancer or Super-enhancer	H3K27ac Fold change	H3K4me1 Fold change	H3K4me3 Fold change
Mesenchymal Stem Cell	chr16:54965-54970	3.16	7.07	IRX5			2.6	4.15	23.9
Mesenchymal Stem Cell	chr16:55505-55510	2.25	6.99			Enhancer	8.75	3.61	2.27
Mesenchymal Stem Cell	chr16:55500-55505	2.01	6.24			Enhancer	6.41	4.15	3.86
Mesenchymal Stem Cell	chr16:55540-55545	1.29	4.11	LPCAT2			1.54	5.61	11.77
Mesenchymal Stem Cell	chr16:55510-55515	1.29	4.03	MMP2			2.07	5.61	23.5
Mesenchymal Stem Cell	chr16:55535-55540	1.02	3.22			Enhancer	6.1	5.98	2.14
Mesenchymal Stem Cell	chr16:55355-55360	1.06	3.01	IRX6			2.47	3.04	11.24
Mesenchymal Stem Cell	chr16:54320-54325	2.56	2.88	IRX3			3.87	6.16	18.62
Mesenchymal Stem Cell	chr16:55530-55535	0.86	2.73			Enhancer	6.63	5.39	2.8
Mesenchymal Stem Cell	chr16:55515-55520	0.74	2.32	MMP2			1.34	3.73	1.75
Mesenchymal Stem Cell	chr16:55310-55315	0.83	2.3			Enhancer	3.36	3.59	2.14
Mesenchymal Stem Cell	chr16:52115-52120	0.73	2.24	LINC00919			1.33	1.24	1.22
Mesenchymal Stem Cell	chr16:55705-55710	0.62	2.16	SLC6A2			1.12	1.79	2.27
Mesenchymal Stem Cell	chr16:54375-54380	1.74	2.14			Enhancer	3.12	5.96	2.8
Mesenchymal Stem Cell	chr16:55600-55605	0.62	2.05	CAPNS2			1.65	2.33	1.75
Mesenchymal Stem Cell	chr16:55315-55320	0.74	2.05			Enhancer	6.31	4.15	1.75
Mesenchymal Stem Cell	chr16:54490-54495	0.89	1.29		rs9921518		1.54	2.88	1.75
		6.22							1.33

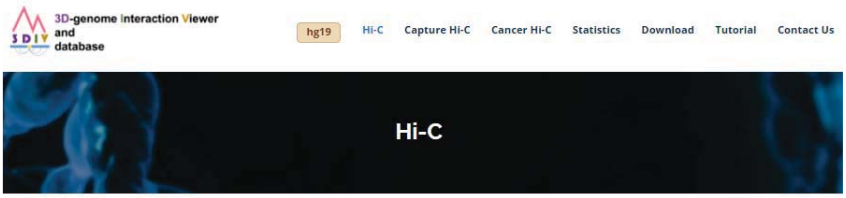
Example : Interaction profile of rs1421085

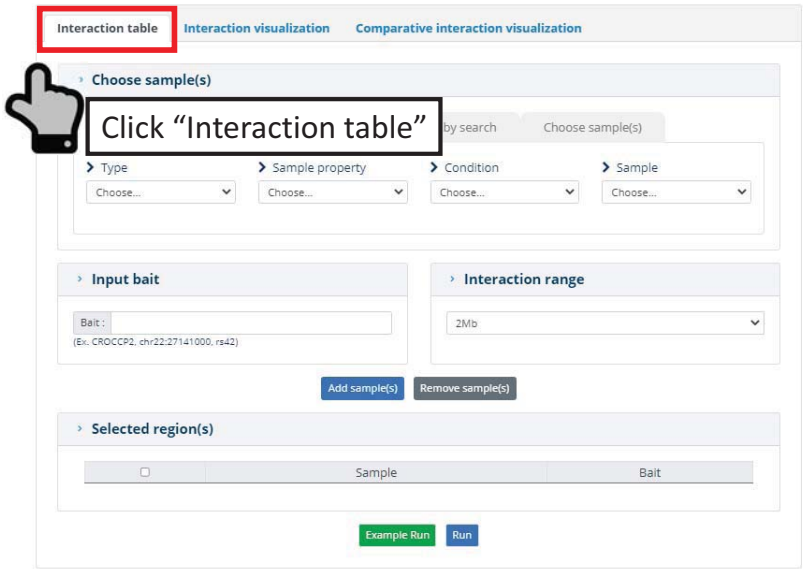
rs1421085 : an obesity variant in FTO gene intron region.
It is well characterized by significant interactions with IRX3 and IRX5 promoters.



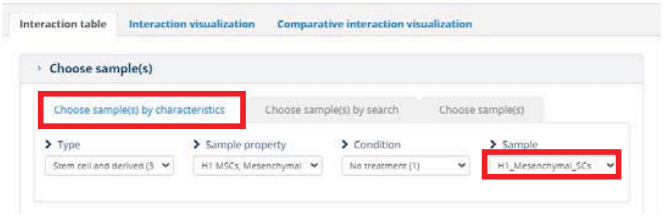
Rask-Andersen et al, Hum. Genet. (2015)

Step 1 : Open Interaction Table Module

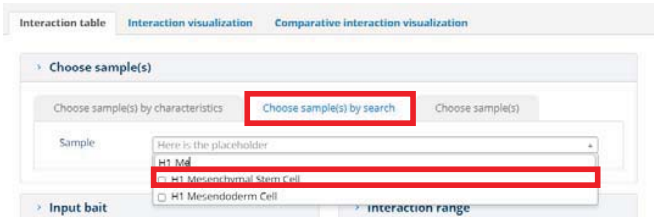




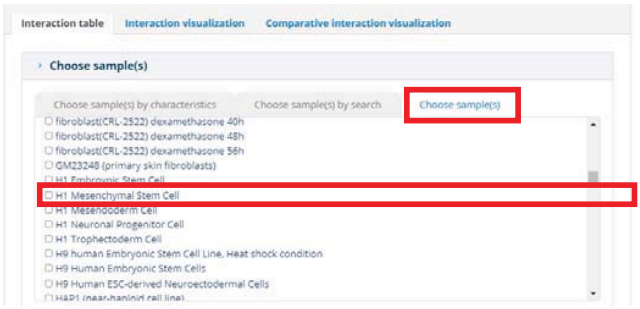
Step 2 : Choose a sample



1) Choose samples with condition

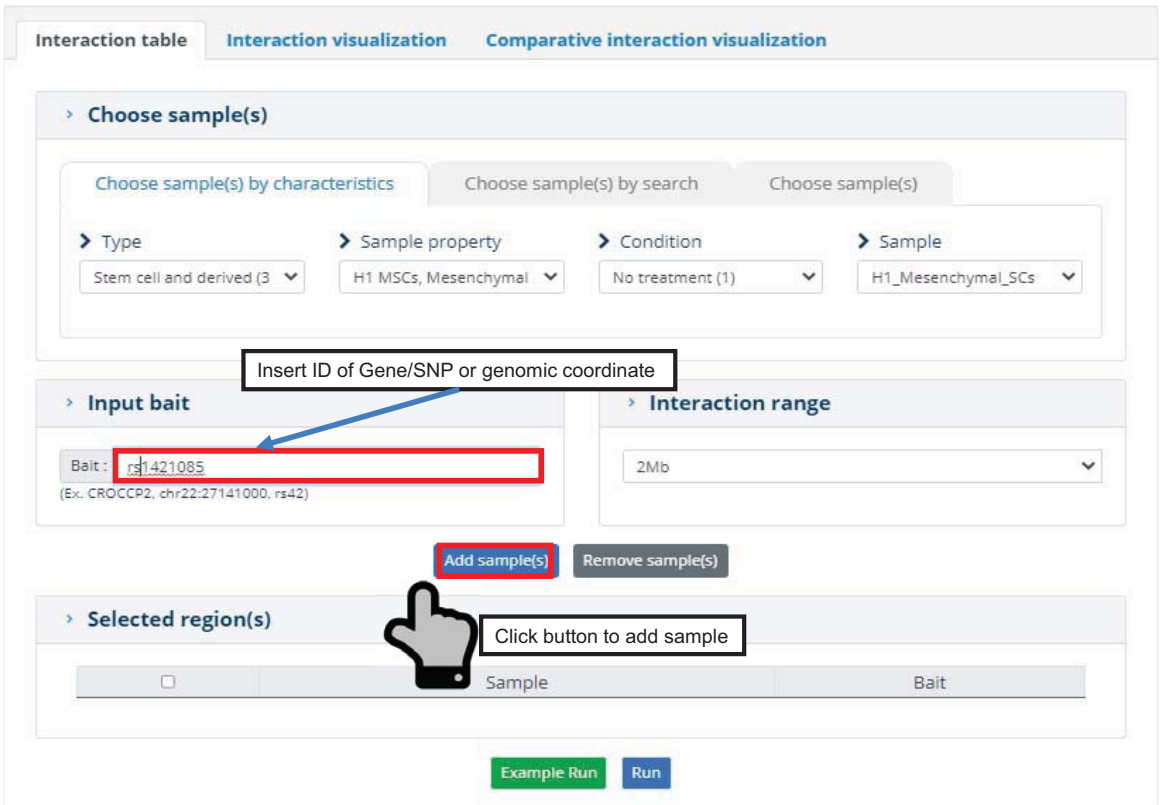


2) Choose samples with searching window

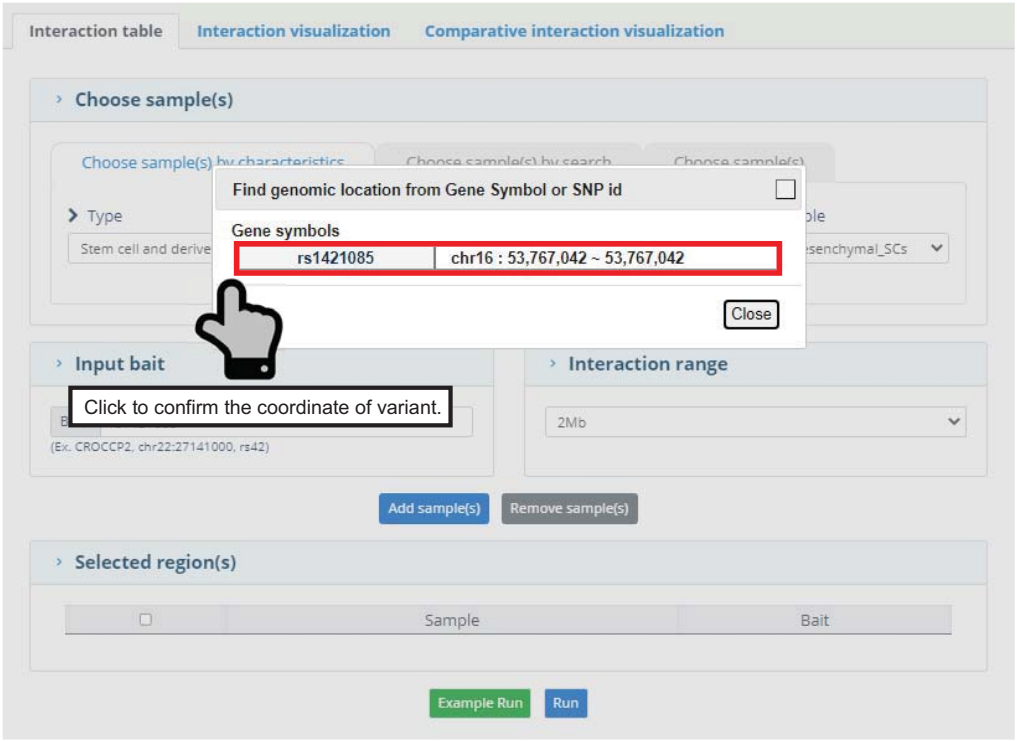


3) Choose samples from the list directly

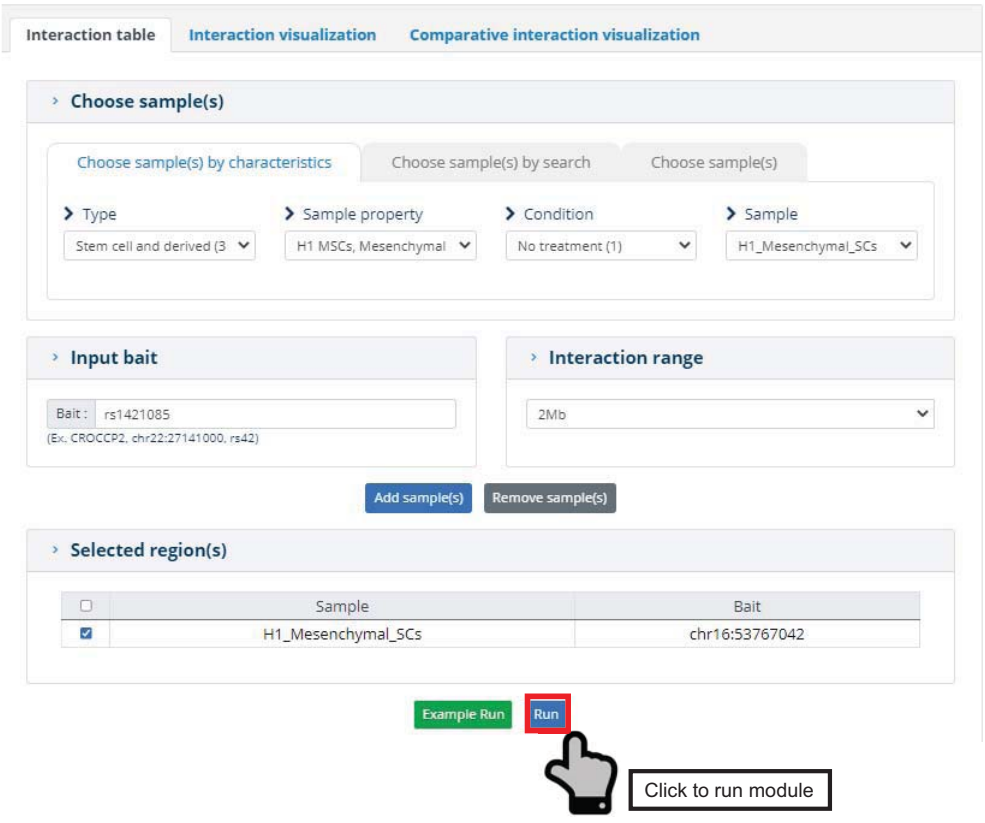
Step 3 : Choose a bait



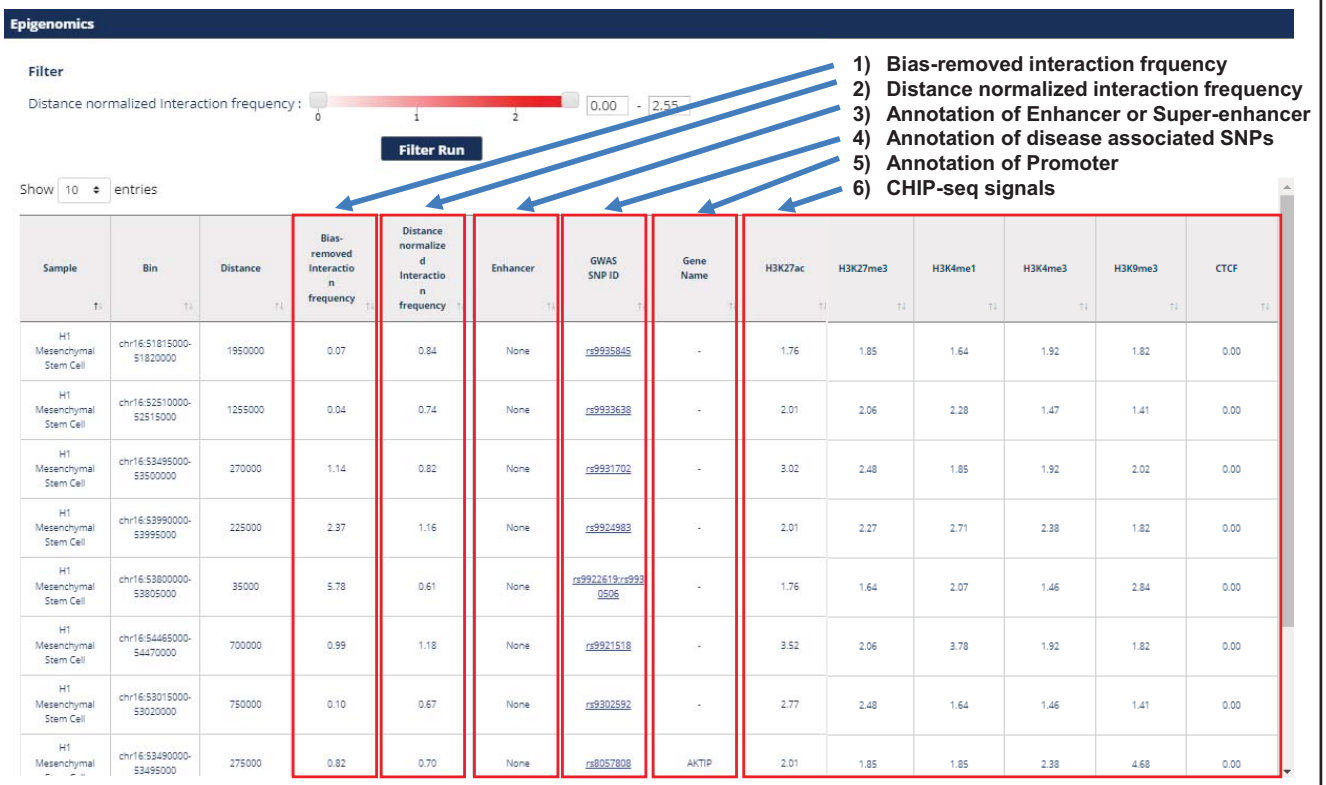
Step 3 : Choose a bait



Step 4 : Run Module

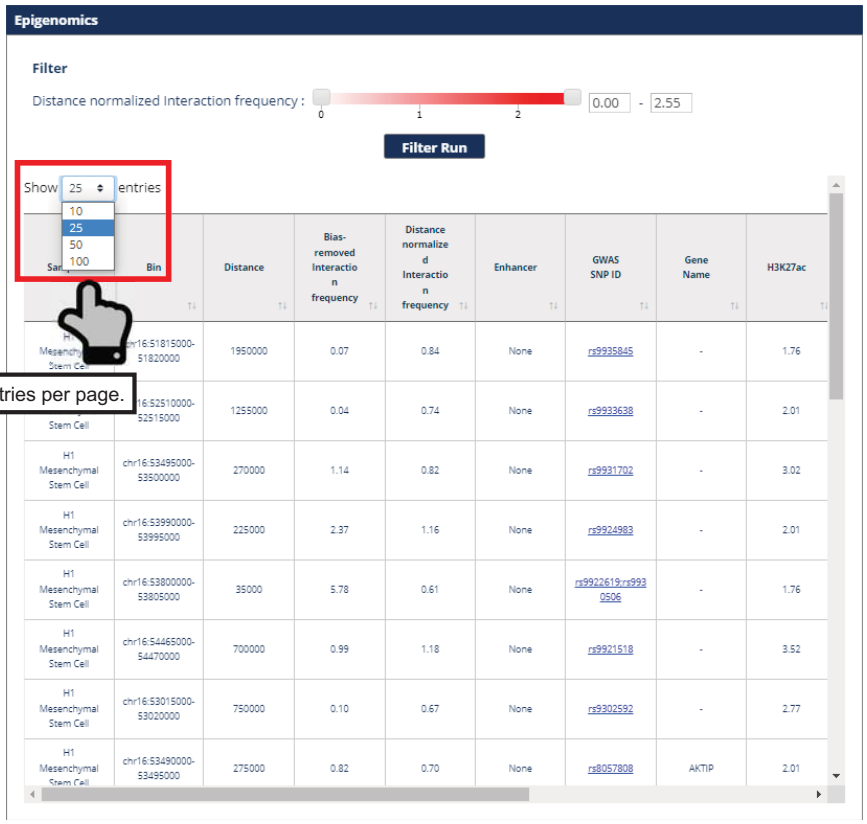


Step 5 : Browse the table



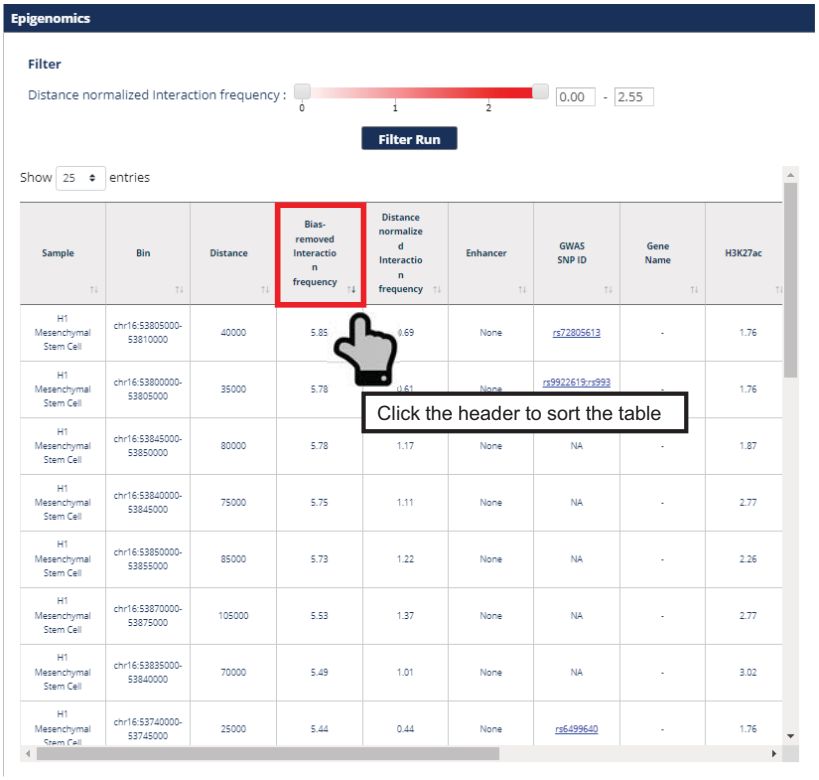
Sample	Bin	Distance	Bias-removed Interaction frequency	Distance normalized Interaction frequency	Enhancer	GWAS SNP ID	Gene Name	H3K27ac	H3K27me3	H3K4me1	H3K4me3	H3K9me3	CTCF
H1 Mesenchymal Stem Cell	chr16:51815000-51820000	1950000	0.07	0.84	None	rs9935845	-	1.76	1.85	1.64	1.92	1.82	0.00
H1 Mesenchymal Stem Cell	chr16:52510000-52515000	1255000	0.04	0.74	None	rs9933638	-	2.01	2.06	2.28	1.47	1.41	0.00
H1 Mesenchymal Stem Cell	chr16:53495000-53500000	270000	1.14	0.82	None	rs9931702	-	3.02	2.48	1.85	1.92	2.02	0.00
H1 Mesenchymal Stem Cell	chr16:53990000-53995000	225000	2.37	1.16	None	rs9924983	-	2.01	2.27	2.71	2.38	1.82	0.00
H1 Mesenchymal Stem Cell	chr16:53800000-53805000	35000	5.78	0.61	None	rs9922619:rs9930506	-	1.76	1.64	2.07	1.46	2.84	0.00
H1 Mesenchymal Stem Cell	chr16:54465000-54470000	700000	0.99	1.18	None	rs9921518	-	3.52	2.06	3.78	1.92	1.82	0.00
H1 Mesenchymal Stem Cell	chr16:53015000-53020000	750000	0.10	0.67	None	rs9302592	-	2.77	2.48	1.64	1.46	1.41	0.00
H1 Mesenchymal	chr16:53490000-53495000	275000	0.82	0.70	None	rs8057808	AKTIP	2.01	1.85	1.85	2.38	4.68	0.00

Step 5a : Adjust the table



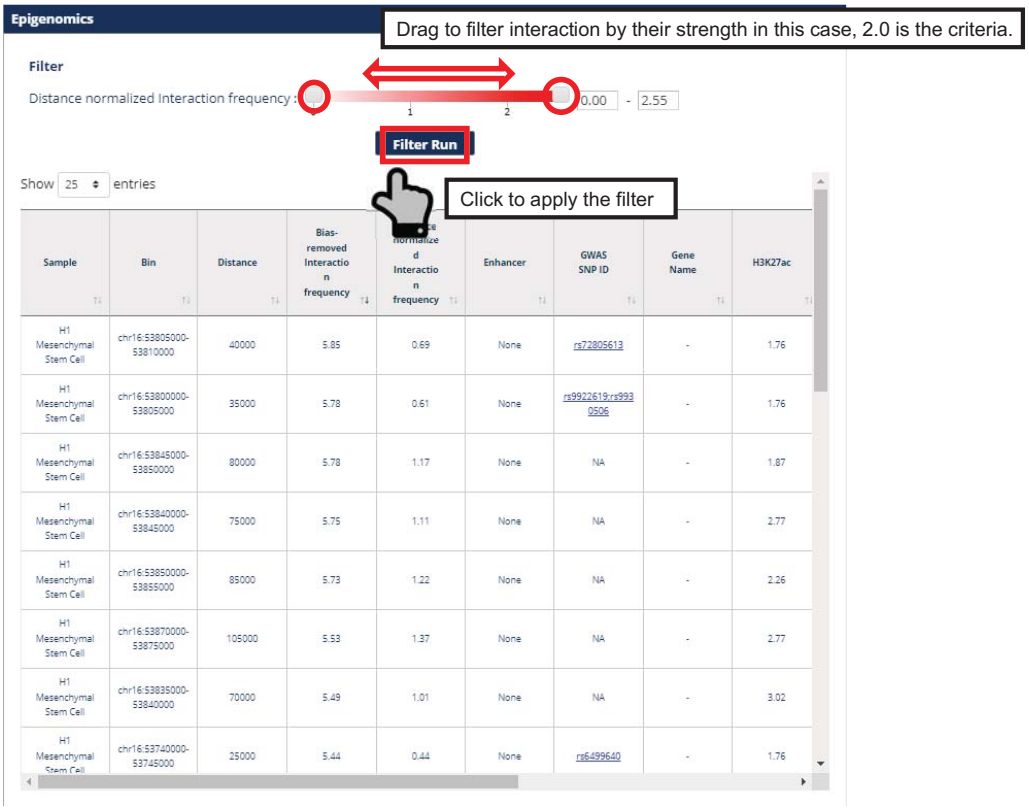
Adjust the number of entries per page.

Step 5b : Sort the interaction table



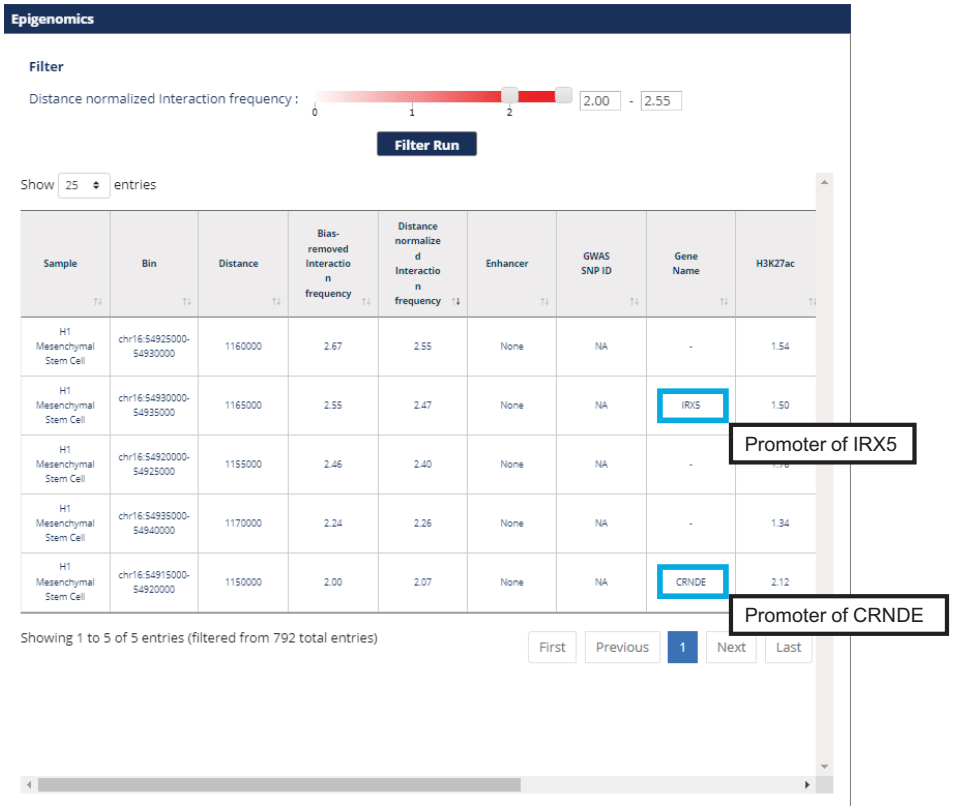
Sample	Bin	Distance	Bias-removed Interaction frequency	Distance normalized Interaction frequency	Enhancer	GWAS SNP ID	Gene Name	H3K27ac
H1 Mesenchymal Stem Cell	chr16:53805000-53810000	40000	5.85	0.69	None	rs72805613	-	1.76
H1 Mesenchymal Stem Cell	chr16:53800000-53805000	35000	5.78	0.61	None	rs9922619:rs993	-	1.76
H1 Mesenchymal Stem Cell	chr16:53845000-53850000	80000	5.78	1.17	None	NA	-	1.87
H1 Mesenchymal Stem Cell	chr16:53840000-53845000	75000	5.75	1.11	None	NA	-	2.77
H1 Mesenchymal Stem Cell	chr16:53850000-53855000	85000	5.73	1.22	None	NA	-	2.26
H1 Mesenchymal Stem Cell	chr16:53870000-53875000	105000	5.53	1.37	None	NA	-	2.77
H1 Mesenchymal Stem Cell	chr16:53835000-53840000	70000	5.49	1.01	None	NA	-	3.02
H1 Mesenchymal Stem Cell	chr16:53740000-53745000	25000	5.44	0.44	None	rs6499640	-	1.76

Step 5c : Filter interaction

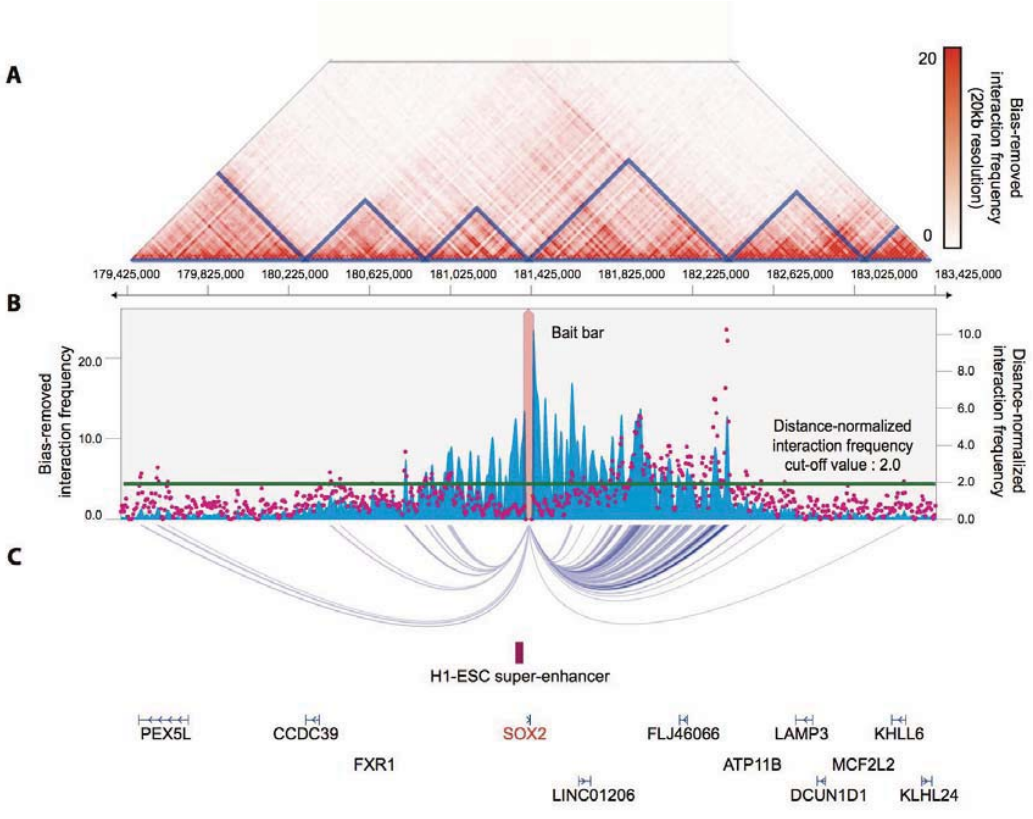


Sample	Bin	Distance	Bias-removed Interaction frequency	Distance normalized Interaction frequency	Enhancer	GWAS SNP ID	Gene Name	H3K27ac
H1 Mesenchymal Stem Cell	chr16:53805000-53810000	40000	5.85	0.69	None	rs72805613	-	1.76
H1 Mesenchymal Stem Cell	chr16:53800000-53805000	35000	5.78	0.61	None	rs9922619:rs9930506	-	1.76
H1 Mesenchymal Stem Cell	chr16:53845000-53850000	80000	5.78	1.17	None	NA	-	1.87
H1 Mesenchymal Stem Cell	chr16:53840000-53845000	75000	5.75	1.11	None	NA	-	2.77
H1 Mesenchymal Stem Cell	chr16:53850000-53855000	85000	5.73	1.22	None	NA	-	2.26
H1 Mesenchymal Stem Cell	chr16:53870000-53875000	105000	5.53	1.37	None	NA	-	2.77
H1 Mesenchymal Stem Cell	chr16:53835000-53840000	70000	5.49	1.01	None	NA	-	3.02
H1 Mesenchymal Stem Cell	chr16:53740000-53745000	25000	5.44	0.44	None	rs6499640	-	1.76

Step 5 : Browse the table

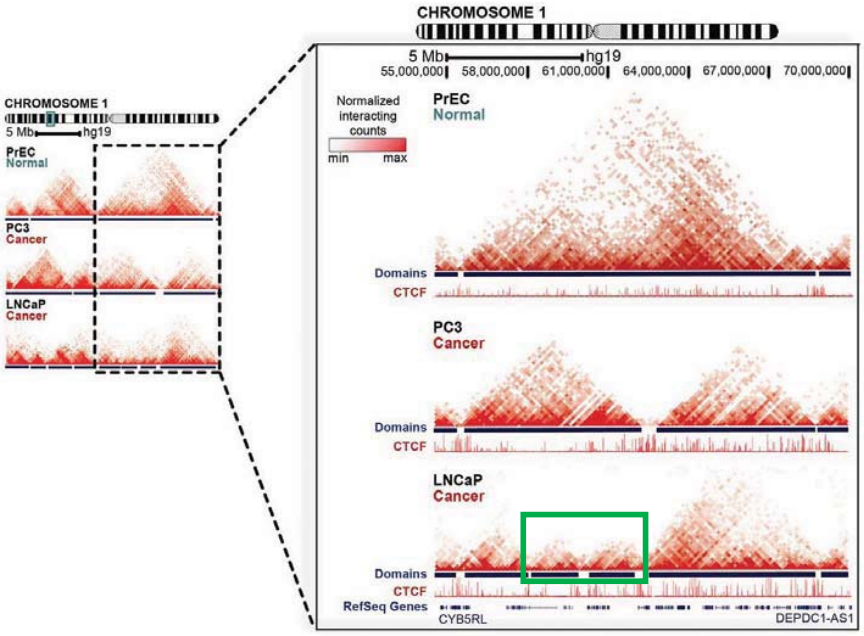


Module 2 : Interaction Visualization



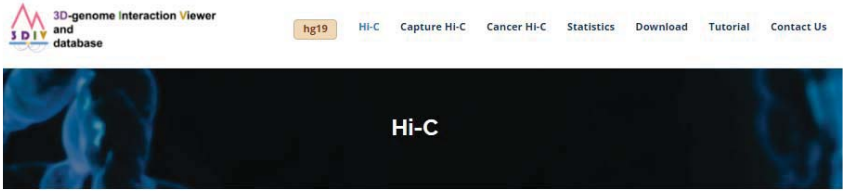
Example : Interaction profile of SOX2

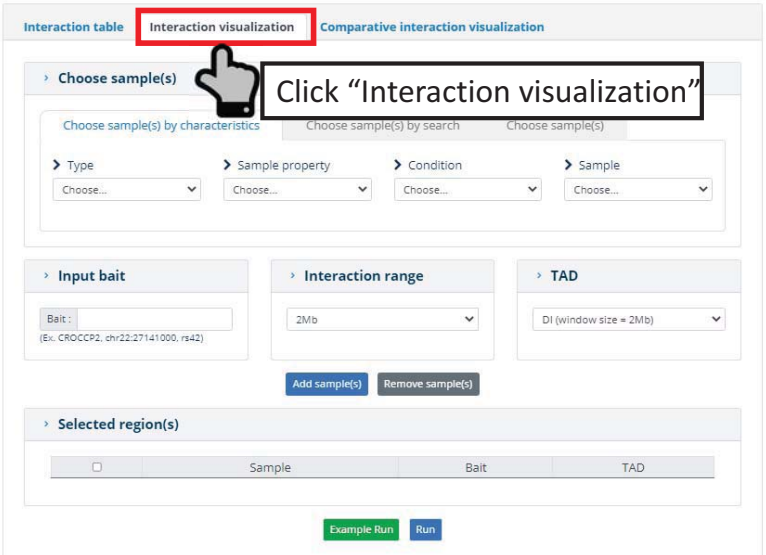
In cancer cells, the genomic structures are degraded into smaller sub-structures.
In this session, we will reproduce this result with 3DIV.



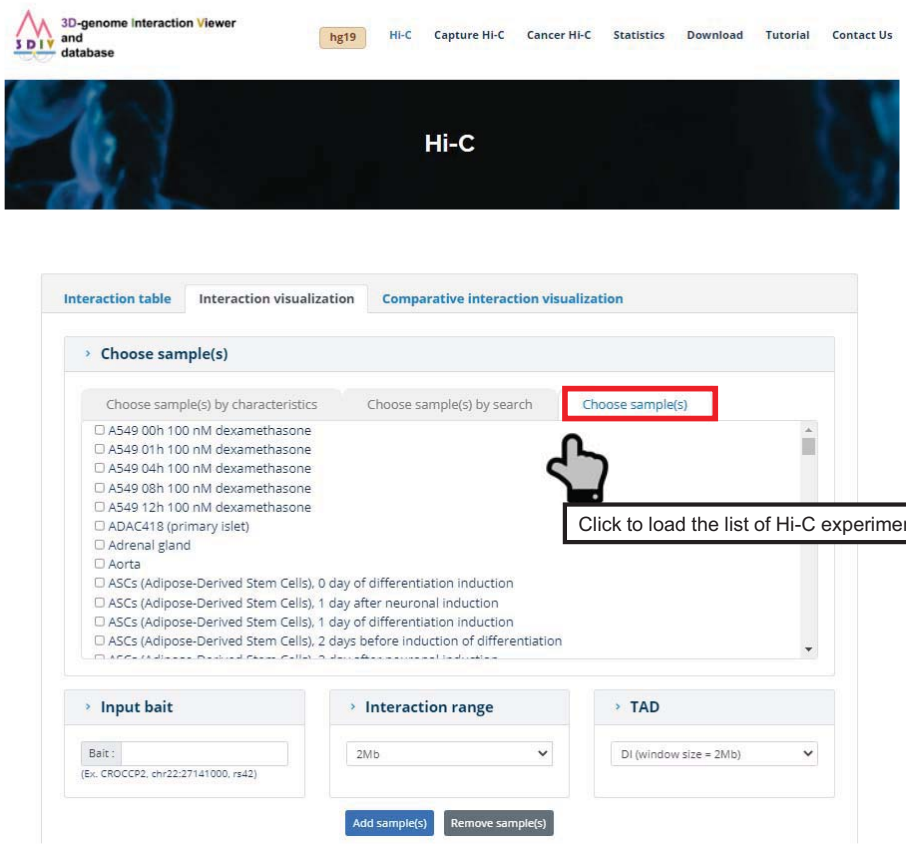
Taberlay et al, *Genome Res.* (2016)

Step 1 : Open Interaction Visualization Module

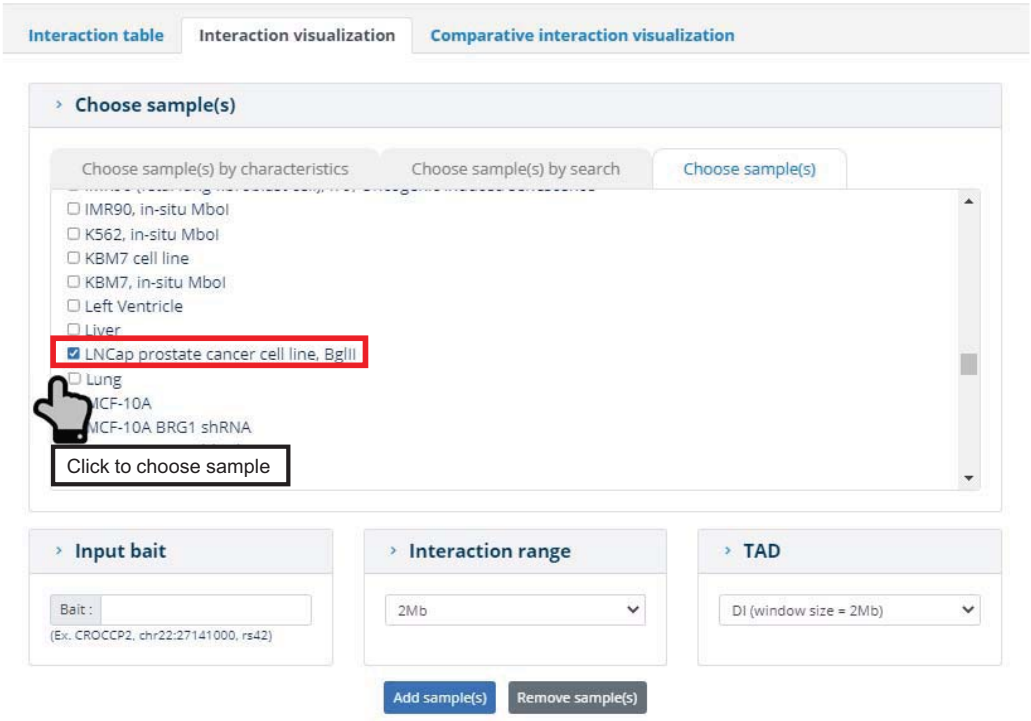




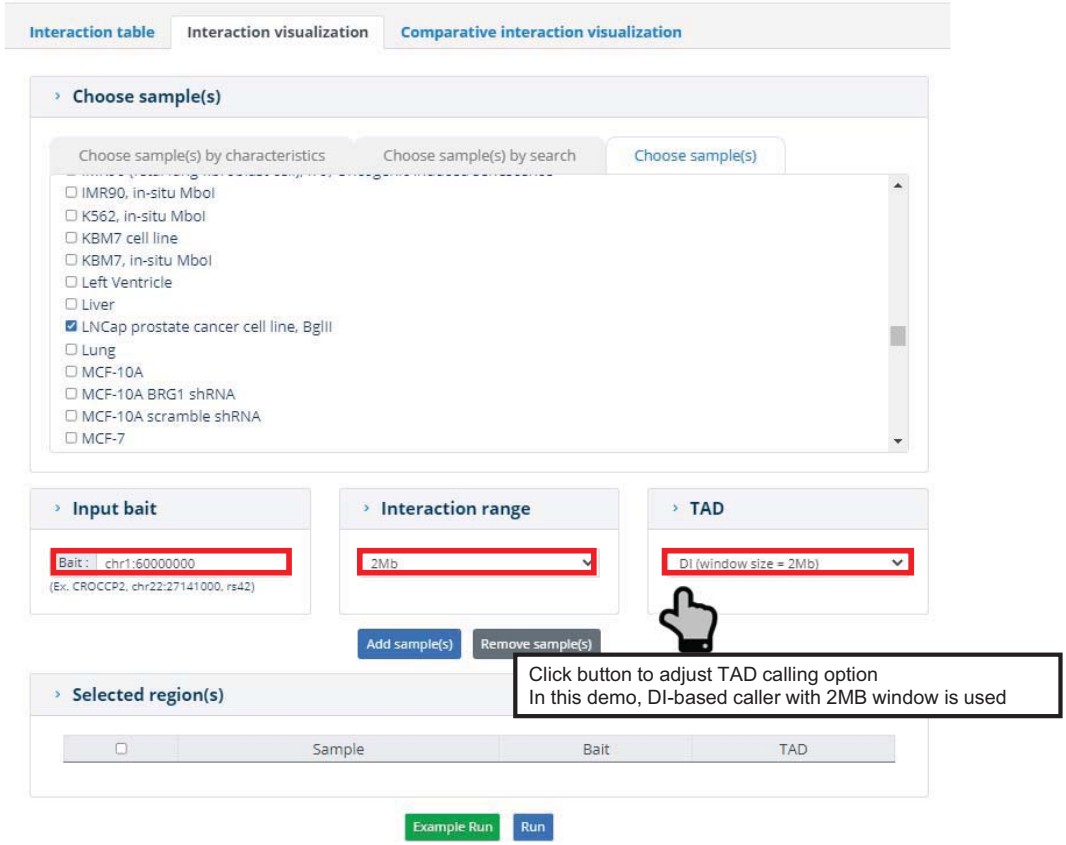
Step 2 : Choose a sample



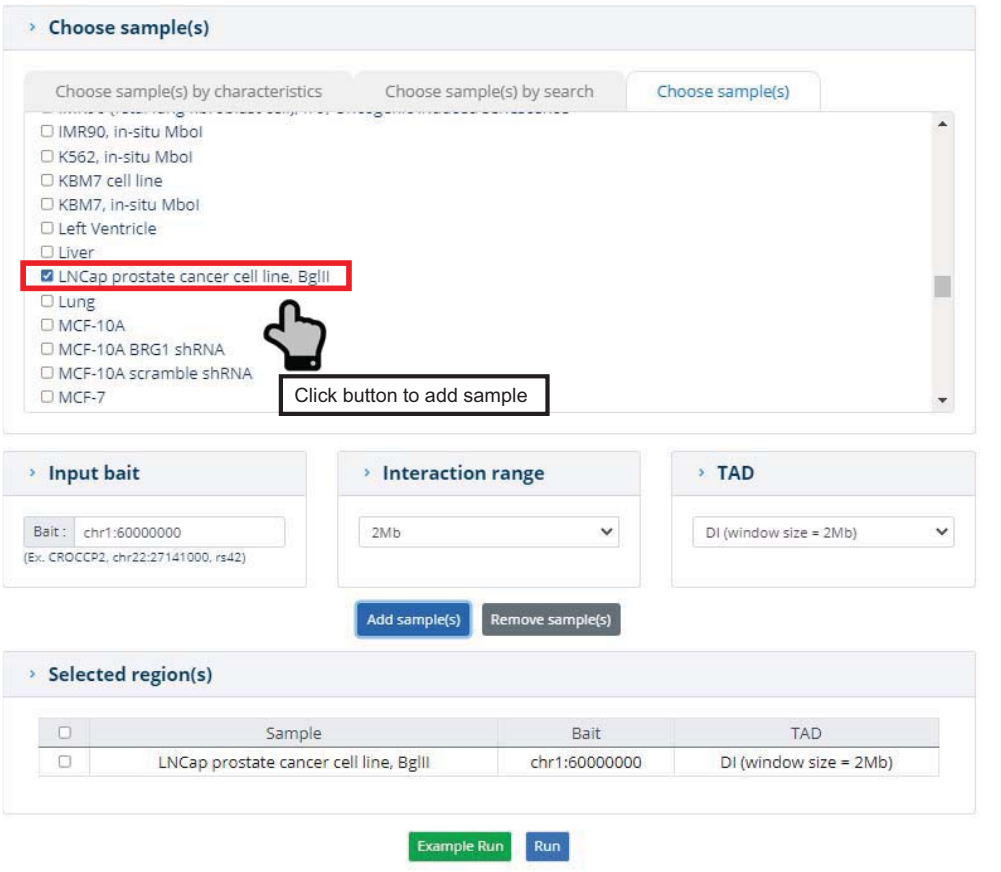
Step 2 : Choose a sample



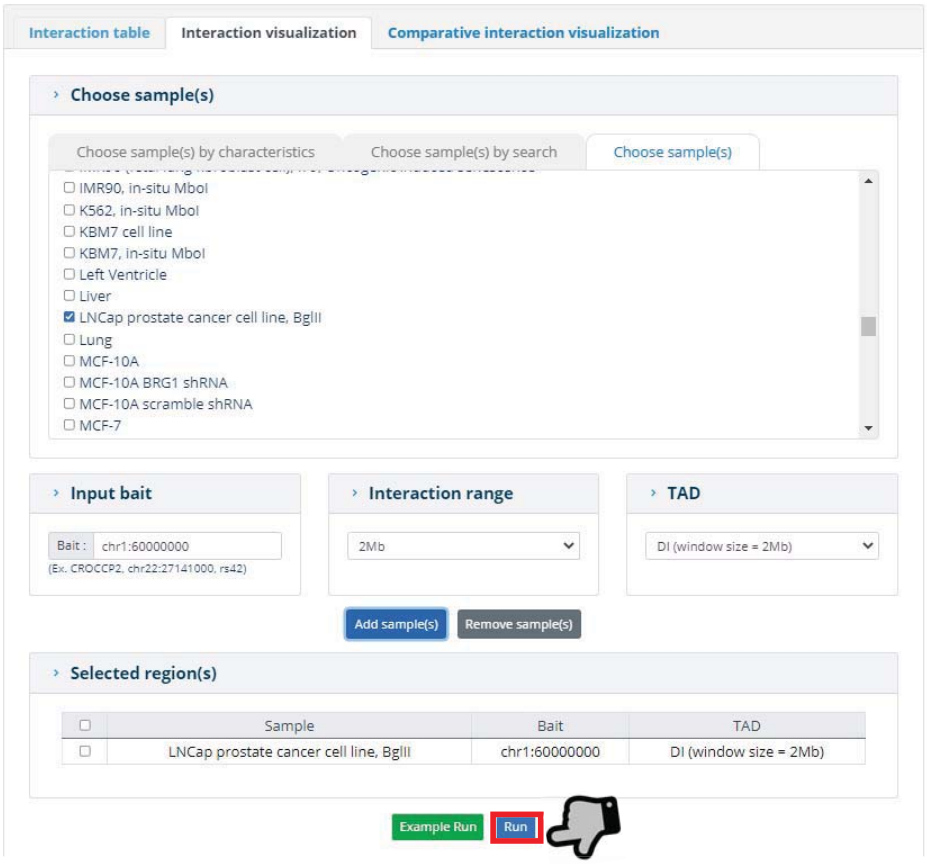
Step 3 : Choose a bait & TAD calling option



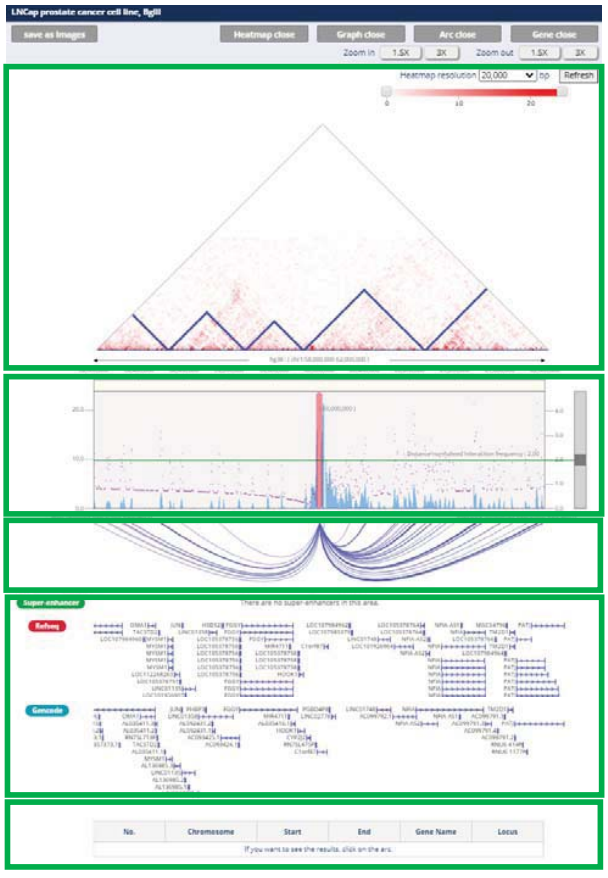
Step 3 : Choose a Bait & TAD calling option



Step 4 : Run Module



Step 5 : Adjust the interaction visualization



Interaction frequency heatmap with topologically associating domains(TAD) annotation.

One-to-all interaction plot

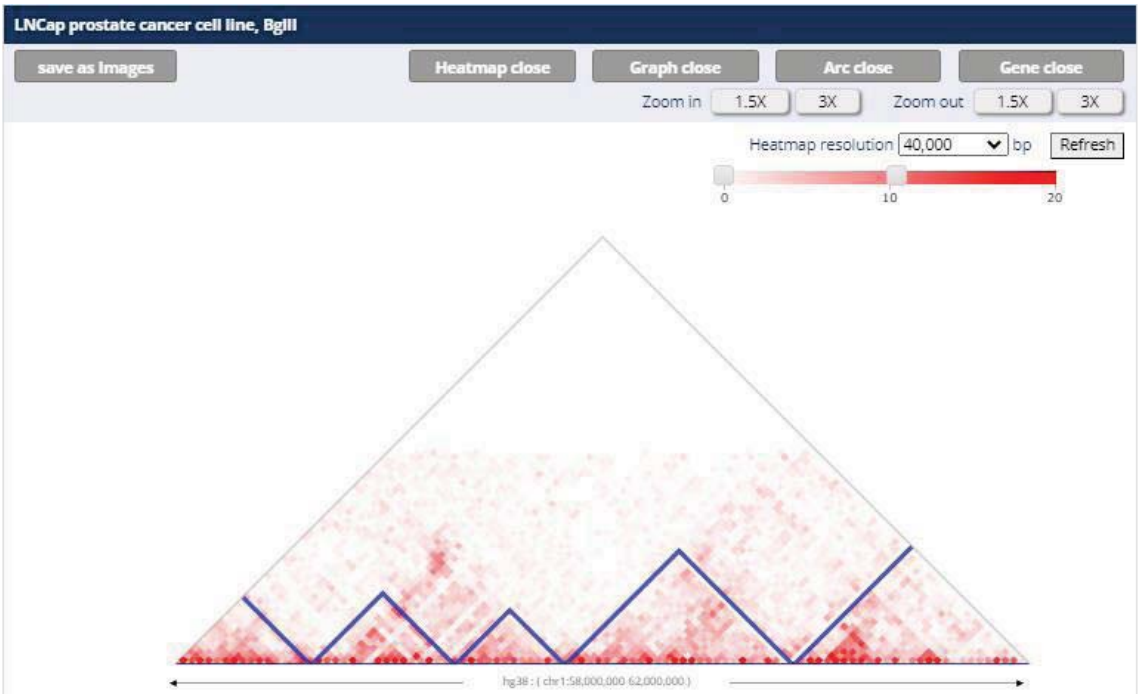
Arc-representation of significant interactions

Gene annotations

Description of selected interaction

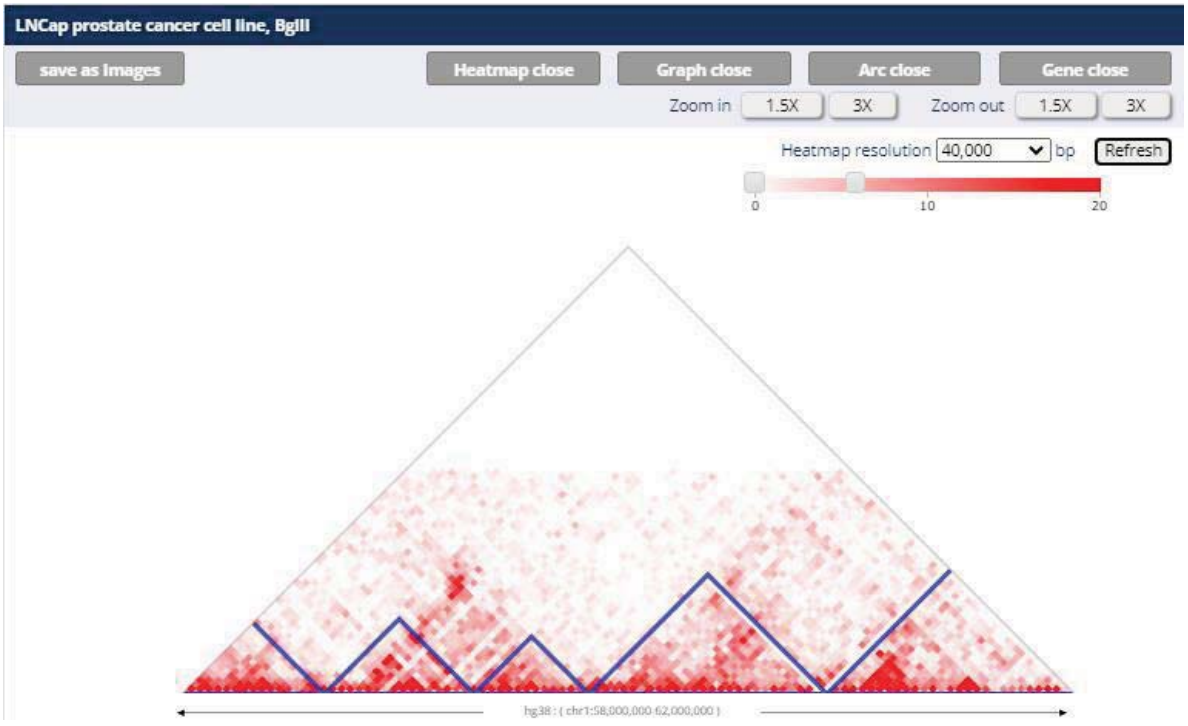
Step 5a : Adjust the heatmap resolution

Step 5a : Adjust the heatmap resolution



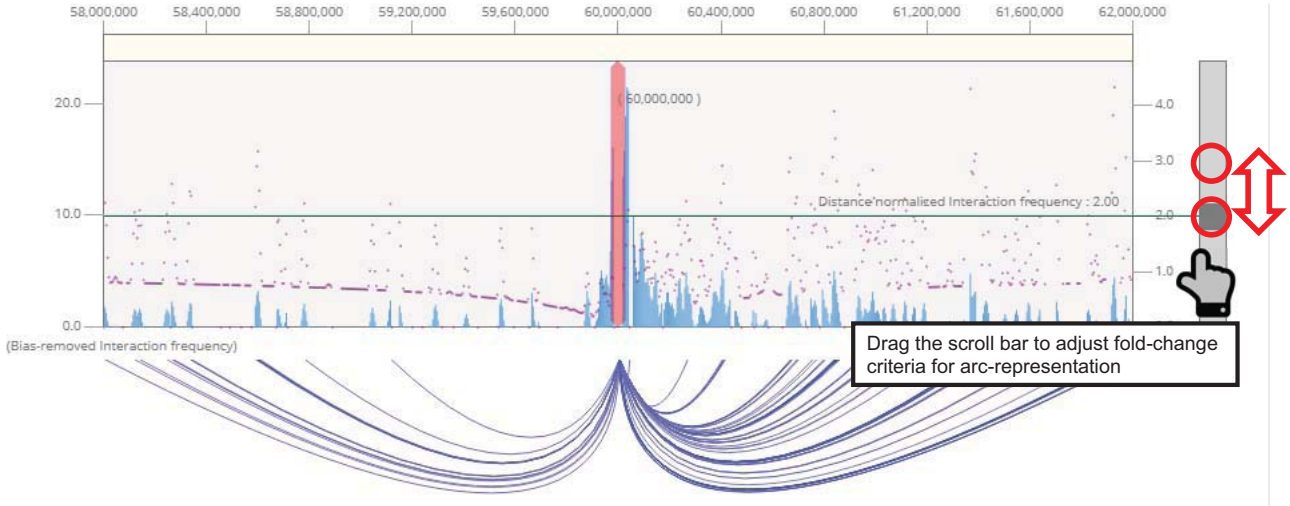
Step 5b : Adjust the heatmap color range

Step 5b : Adjust the heatmap color range

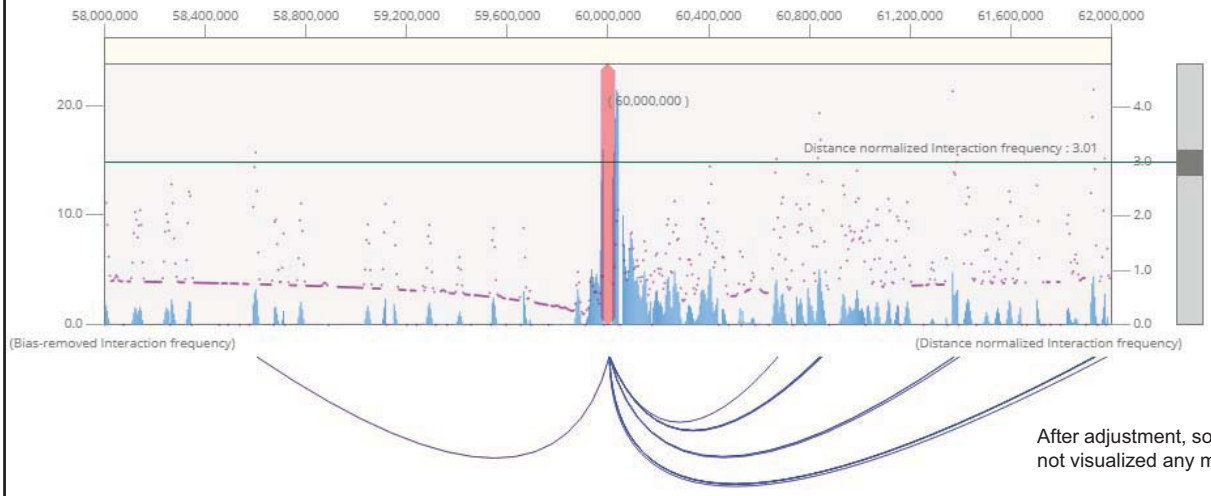


Step 5 : Adjust the interaction visualization

Adjust the fold-change criteria

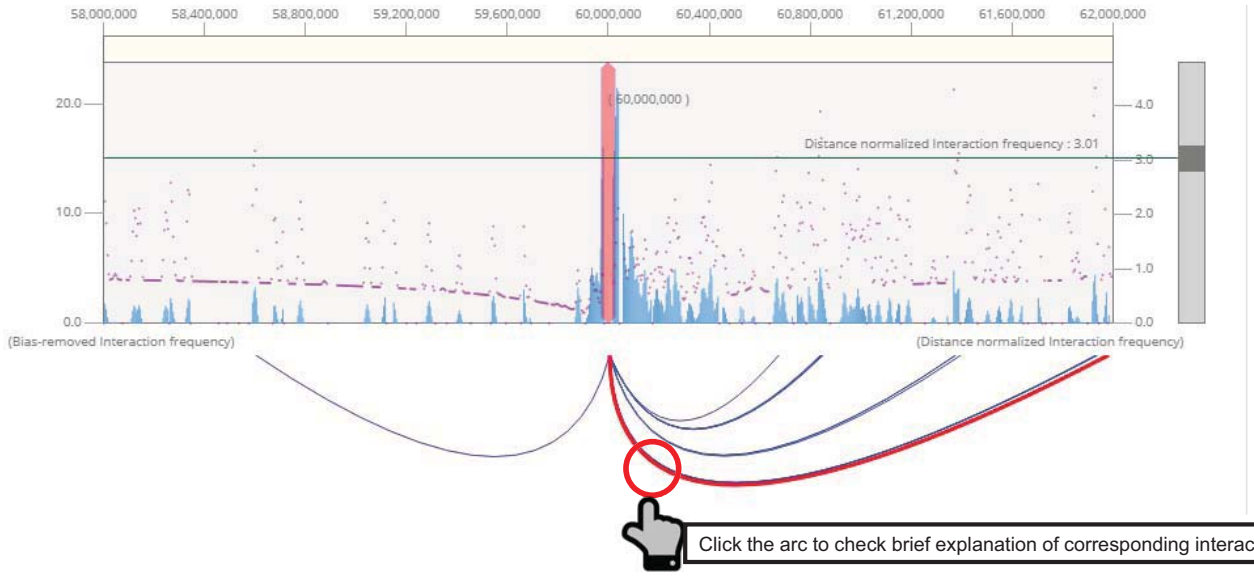


Adjust the fold-change criteria



After adjustment, some arcs are not visualized any more.

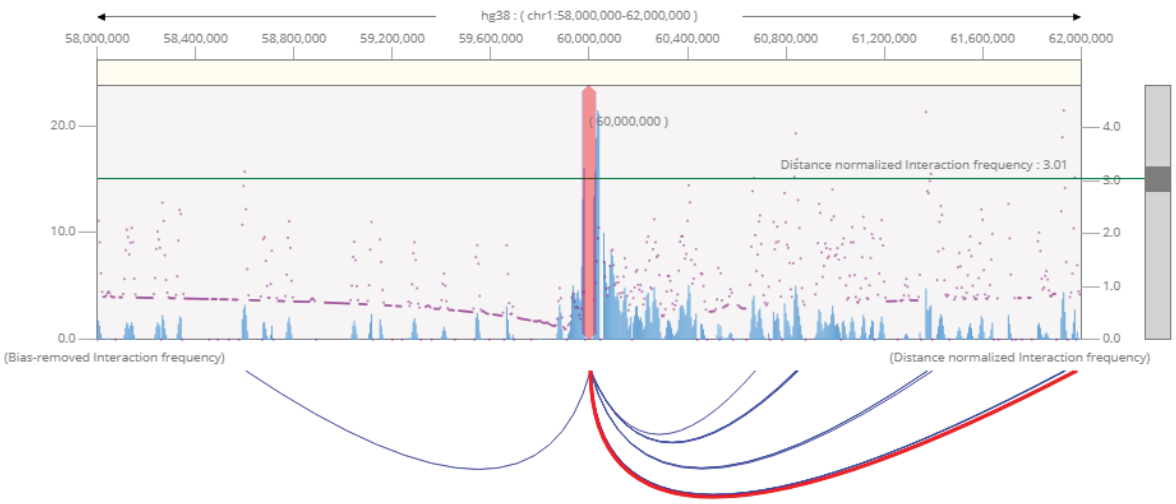
Description of identified interactions



Click the arc to check brief explanation of corresponding interaction

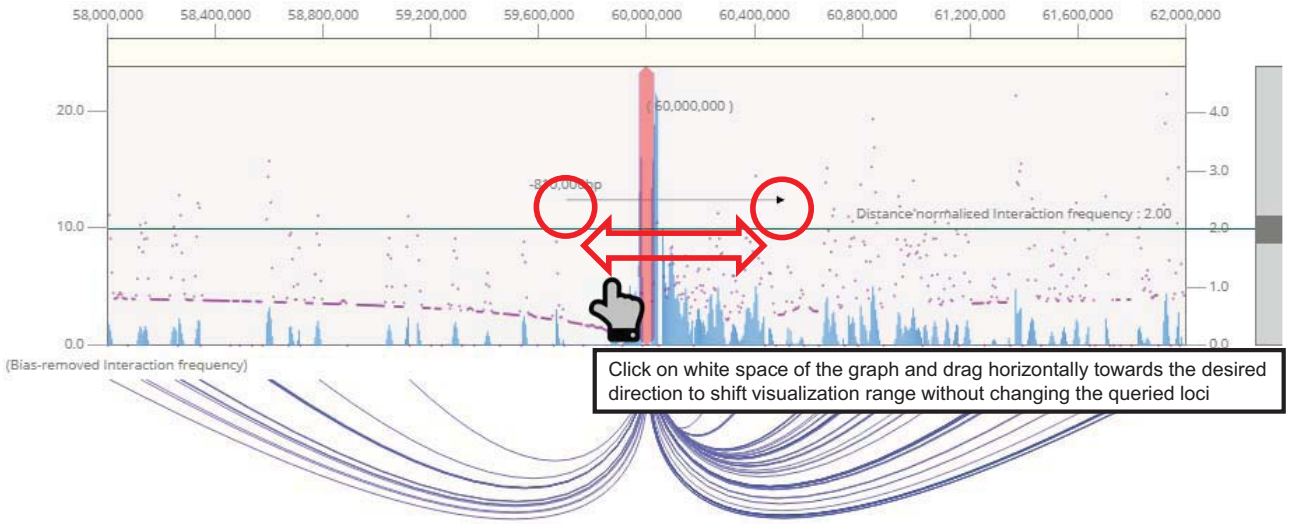
No.	Chromosome	Start	End	Gene Name	Locus
If you want to see the results, click on the arc.					

Description of identified interactions

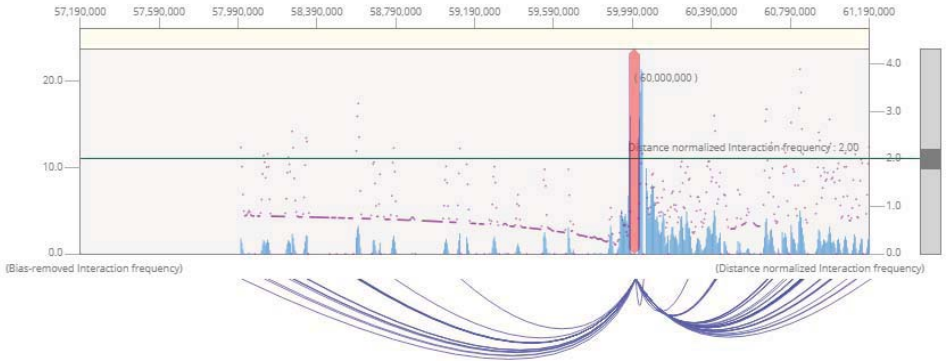


No.	Chromosome	Start	End	Gene Name	Locus
1	chr1	61975000	61980000		

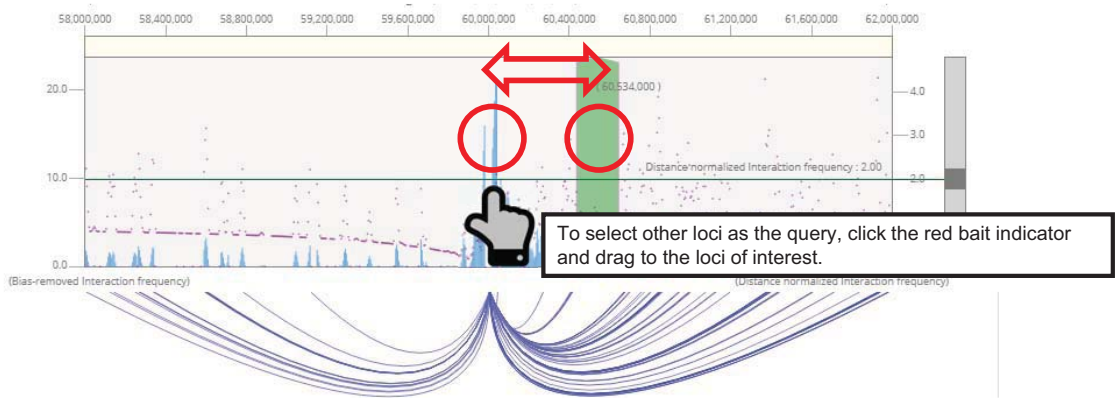
Browse interaction frequency w/o change the bait



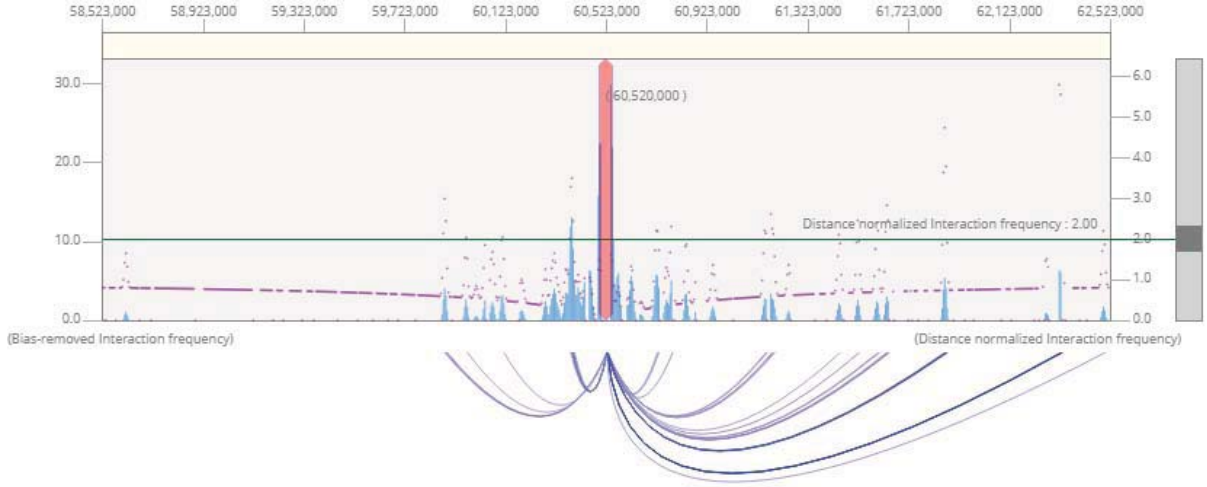
Browse interaction frequency w/o change the bait



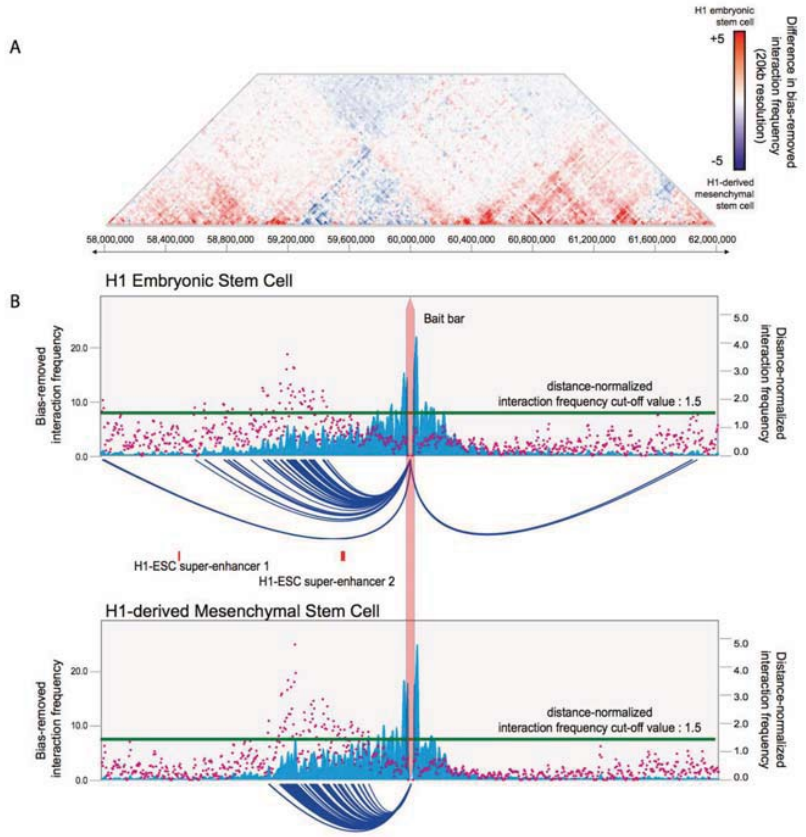
Adjust bait without resubmission



Adjust bait without resubmission

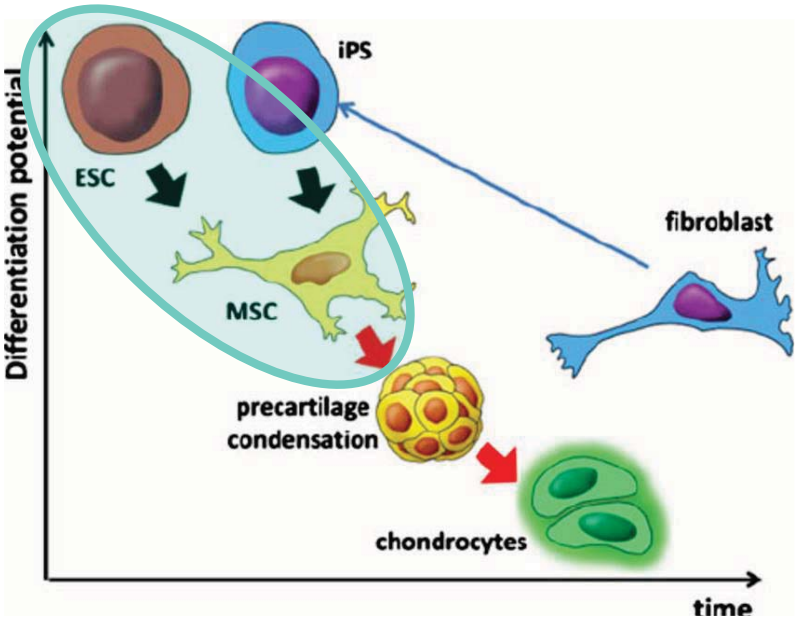


Module 3 : Comparative Visualization



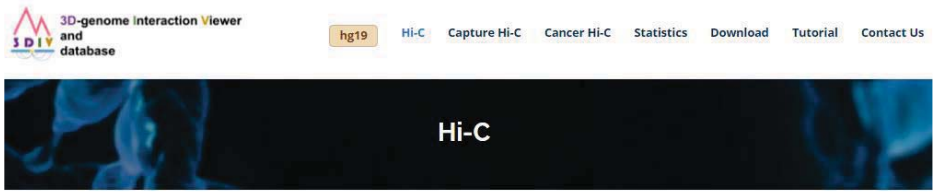
Example : Interaction change during differentiation

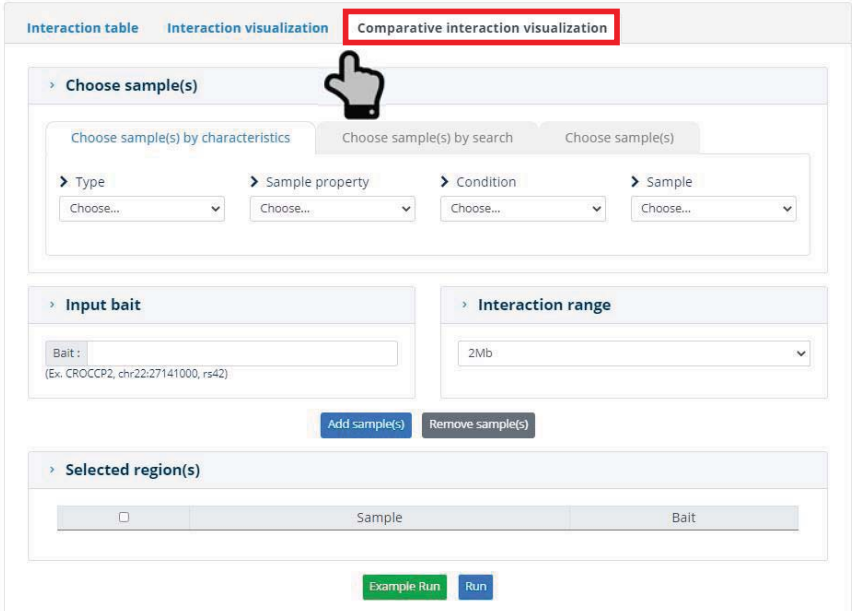
During the differentiation, the interaction profile is dramatically changed.
In this session, we will compare the interaction profile of ESC and MSC.
ESC : Embryonic Stem Cell, MSC : Mesenchymal Stem Cell



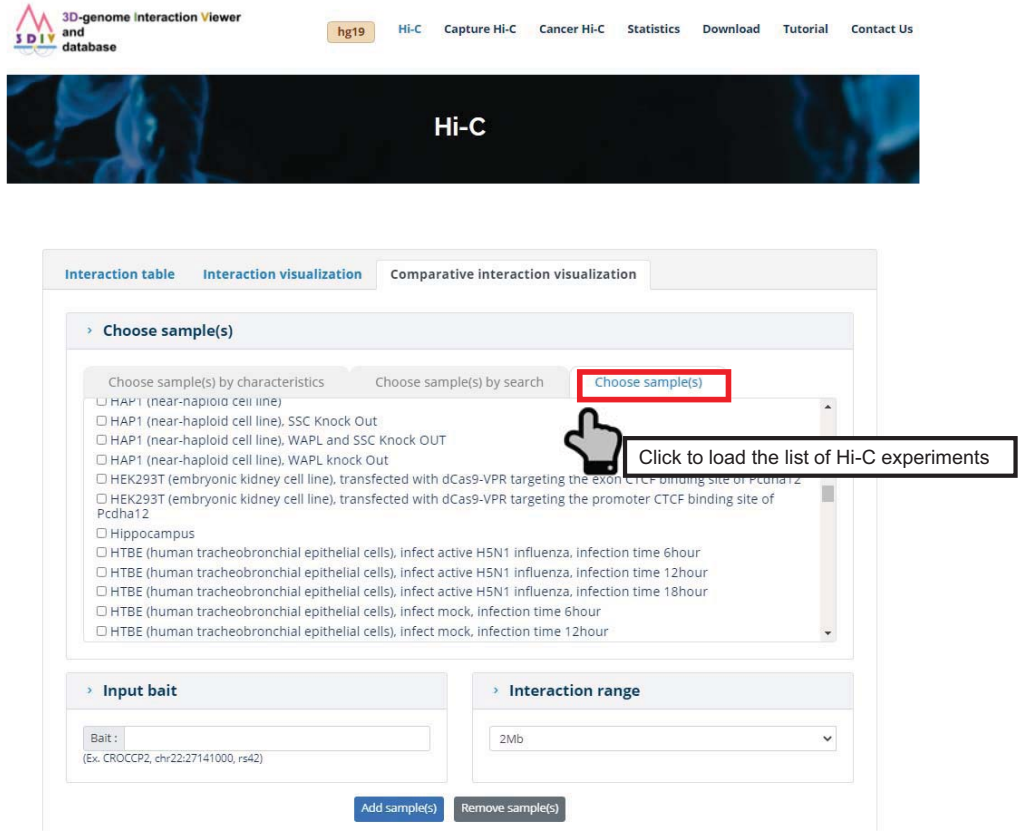
Gadjanski et al, Stem Cell Rev. Rep. (2012)

Step 1 : Open Comparative visualization Module

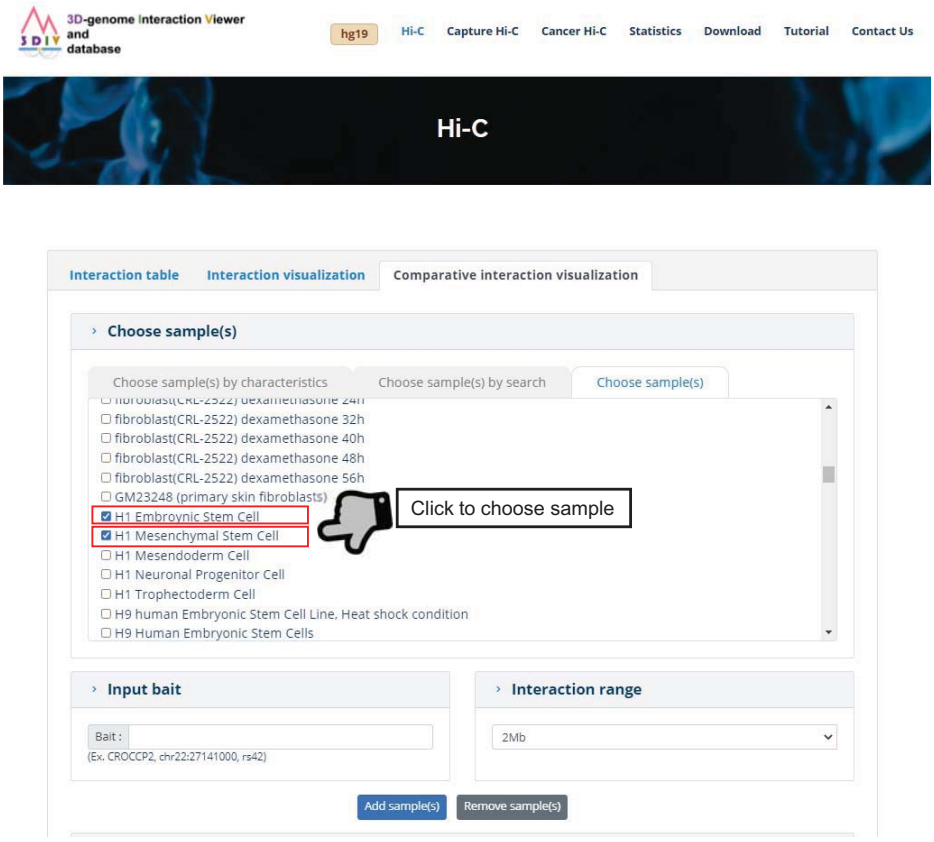




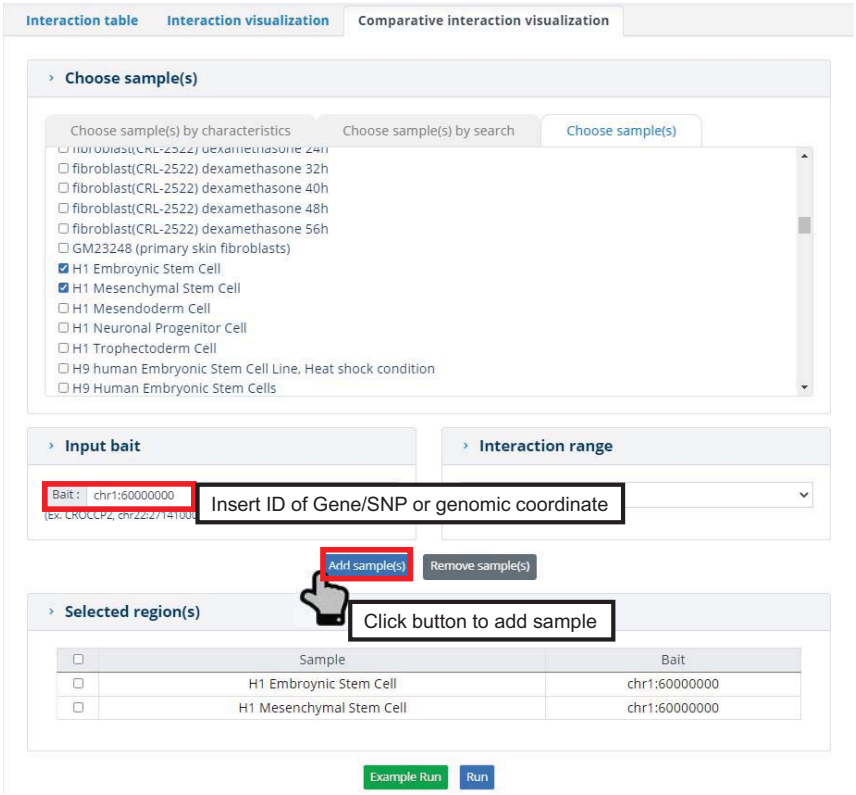
Step 2 : Choose a sample



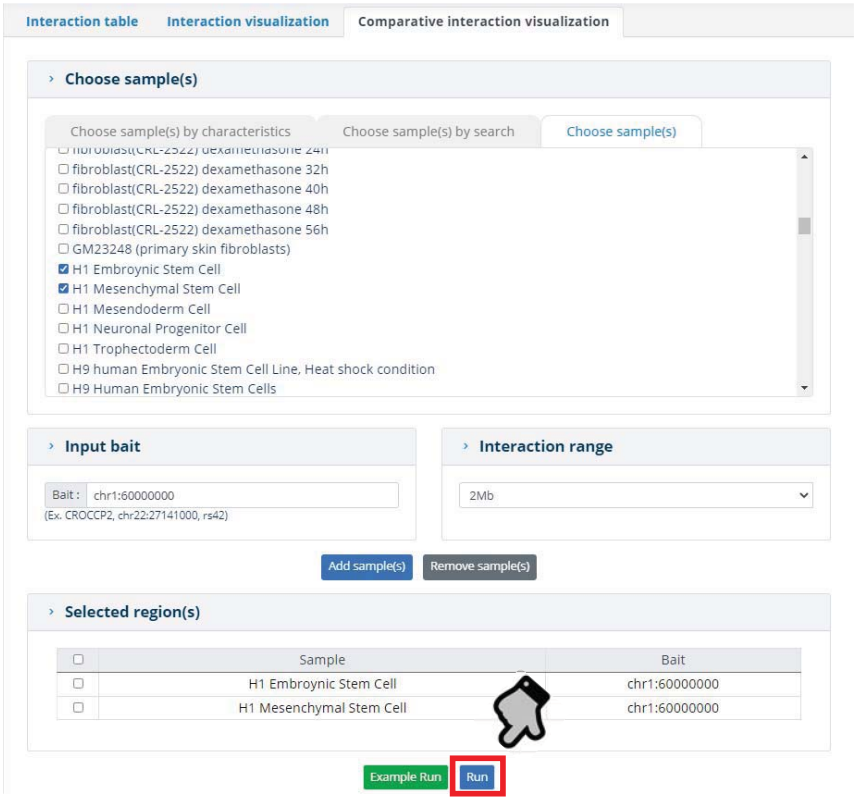
Step 2 : Choose a sample



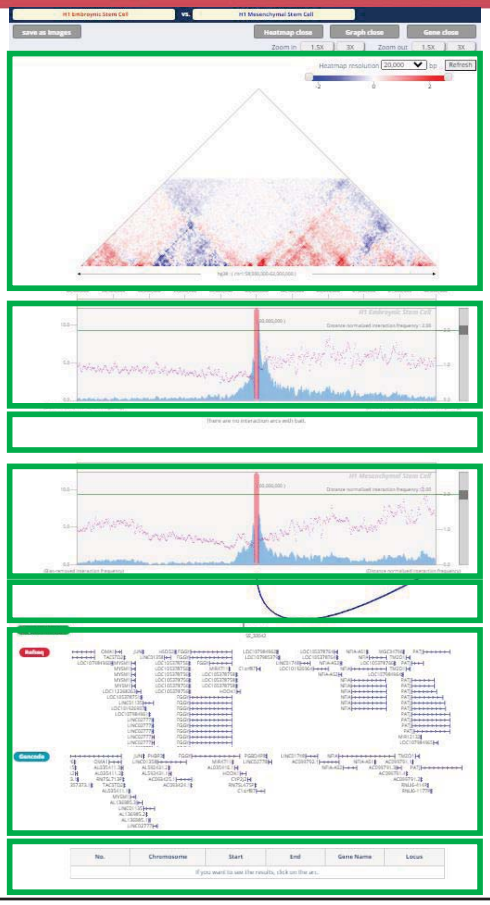
Step 3 : Choose a Bait



Step 4 : Run Module



Step 5 : Adjust comparative heatmap



Comparative heatmap of interaction frequency between 1st and 2nd samples.

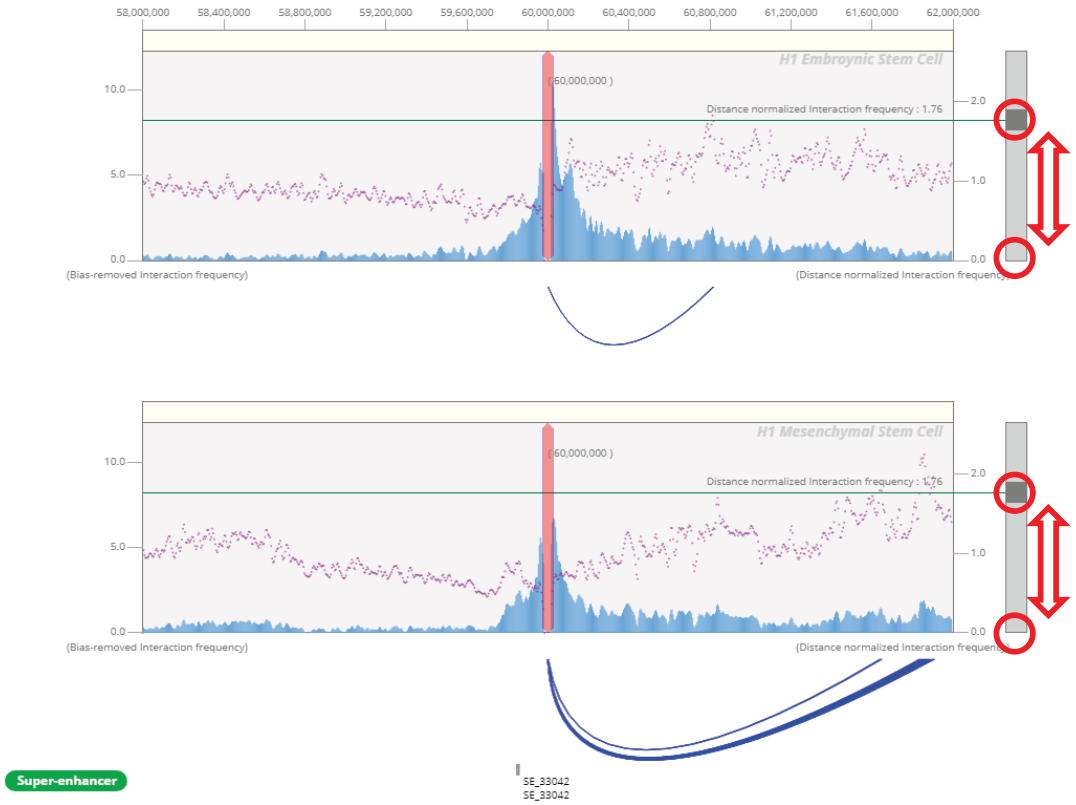
Arc-representation of significant interactions in 1st sample

Arc-representation of significant interactions in 2nd sample

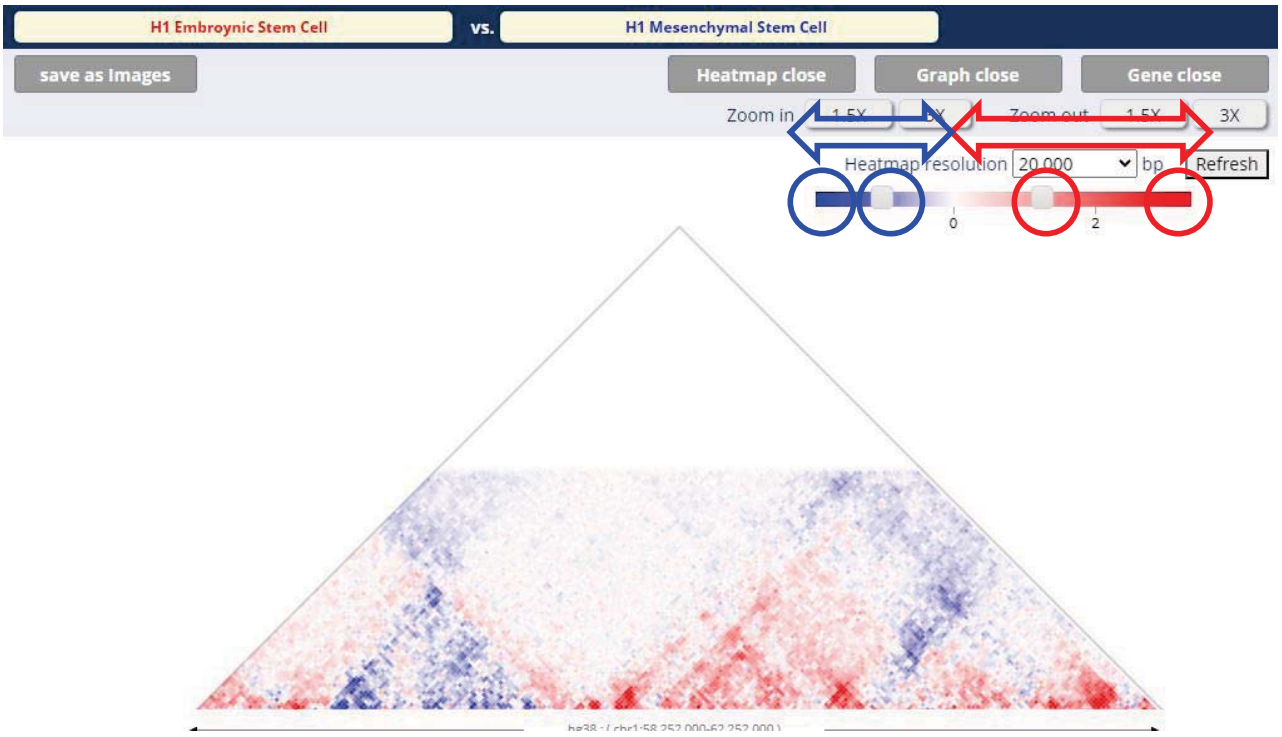
RefSeq Genes and super enhancer annotations

Description of selected interaction

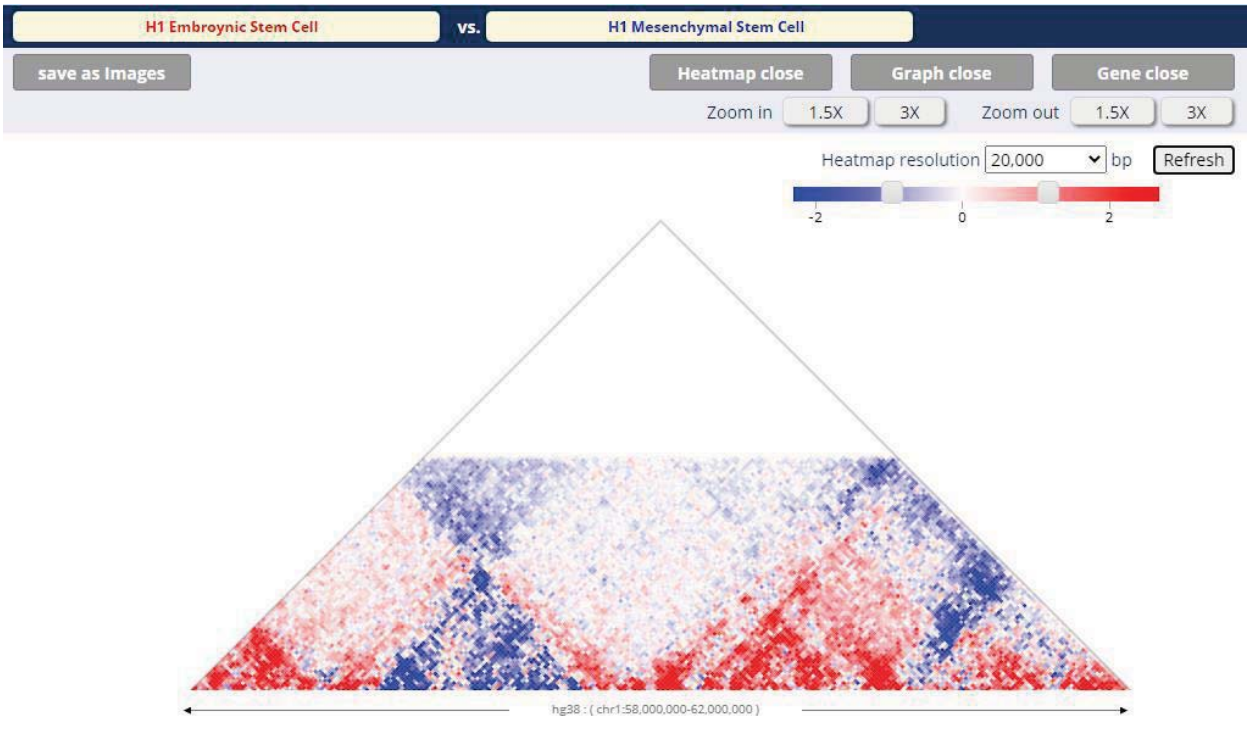
Step 5a : Synchronized criteria change



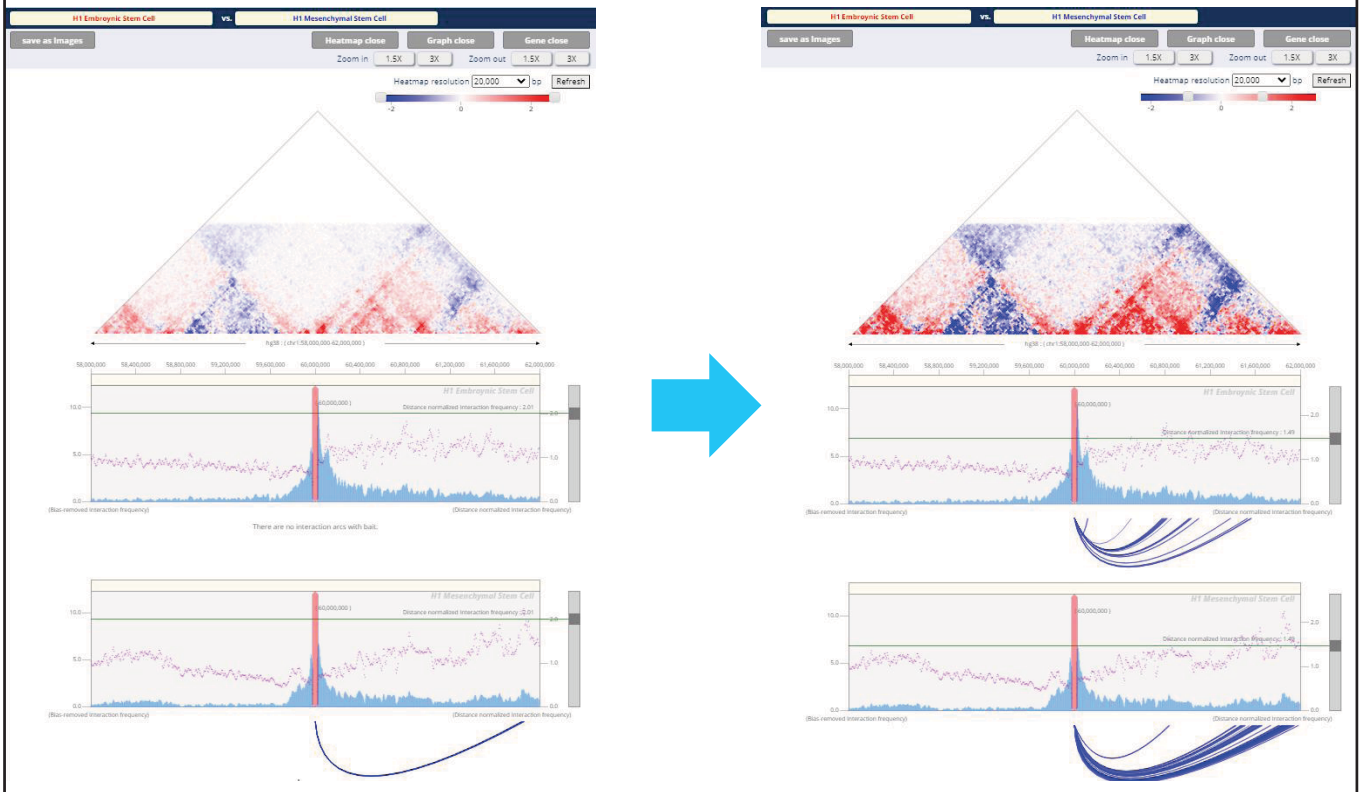
Step 5b : Adjust the heatmap color range



Step 5b : Adjust the heatmap color range

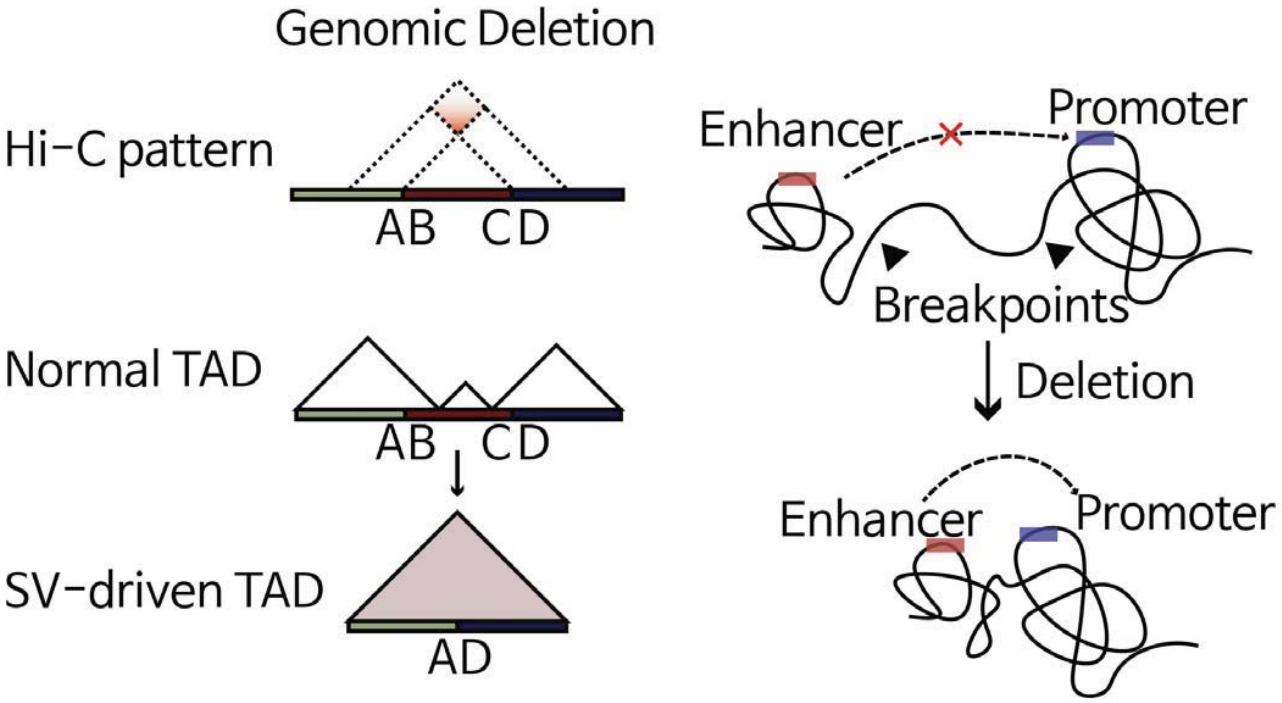


Step 5 : Adjust comparative heatmap



Cancer Hi-C Analysis

The impact of large scale structural variations to cancer 3D genome

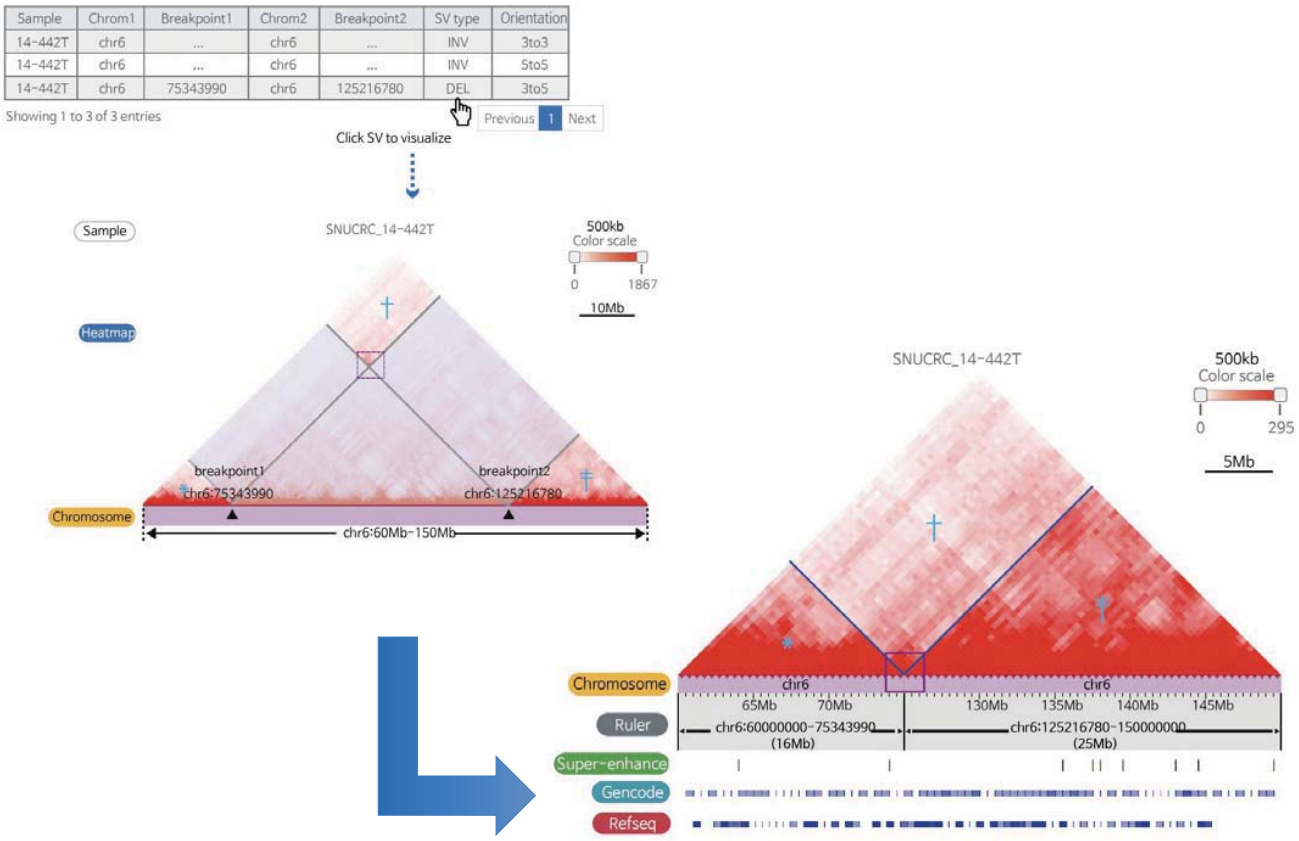


Interactively visualize and simulate the impact of structural variations to cancer 3D genome

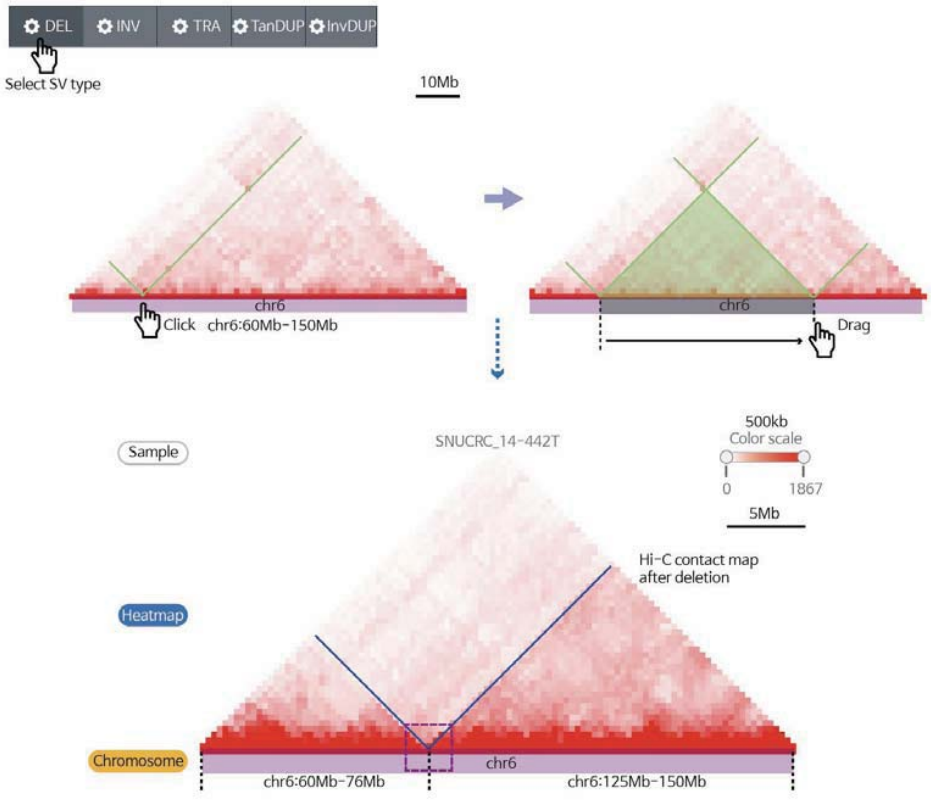
Problem statement
1. Frequent genomic rearrangements in cancer alters 3D genome
2. Abberant gene expression based on rewired regulatory elements
3. Requires appropriate visualization tools and processed data

Resolving issue
1. Collection of large cancer/normal Hi-C and pcHi-C data
2. Visualization of cancer 3D genome
3. Hi-C contact map manipulation to examine impact of SVs

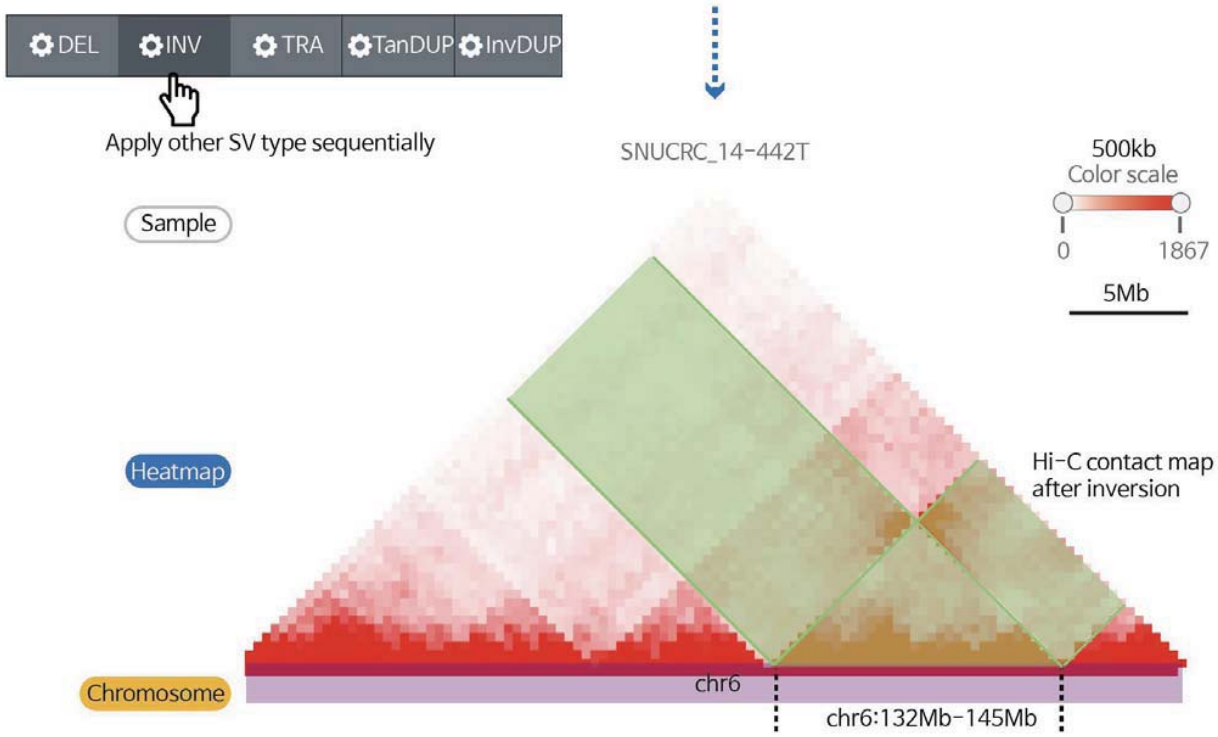
Module I. Pre-called SV and 3D genome



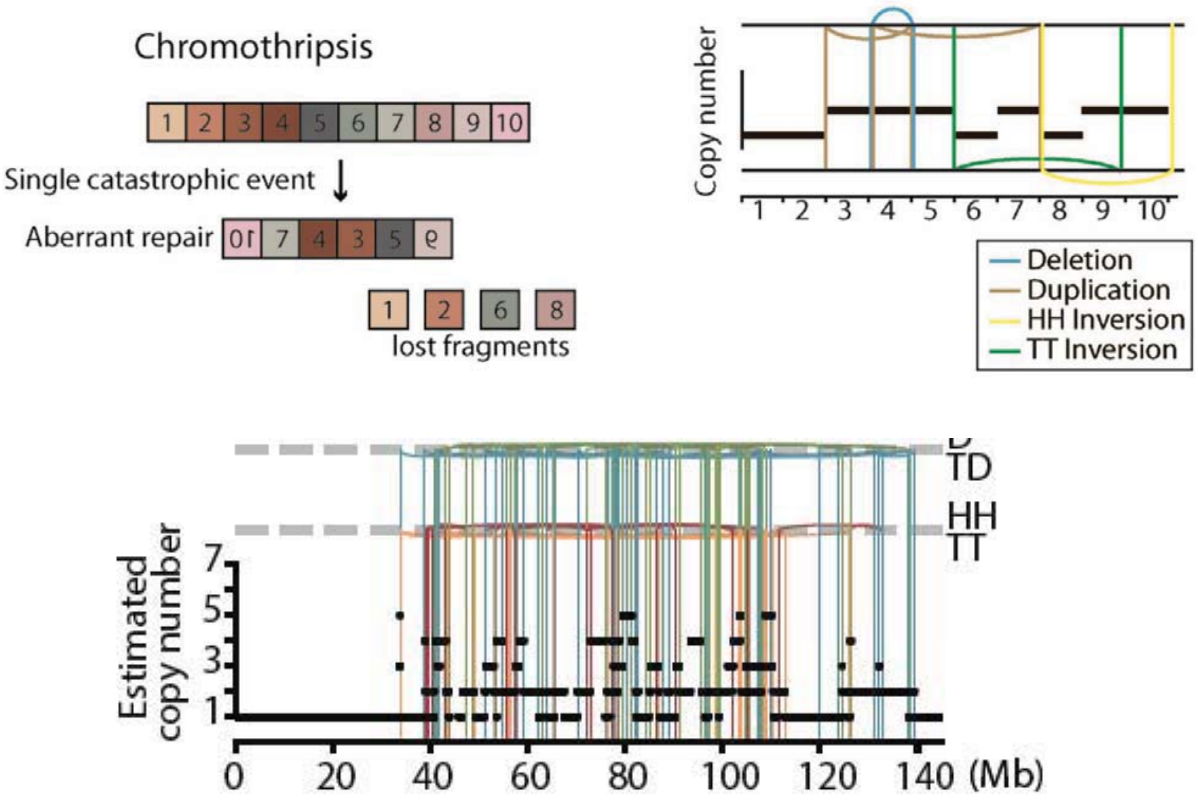
Module II. Interactive 3D genome manipulation



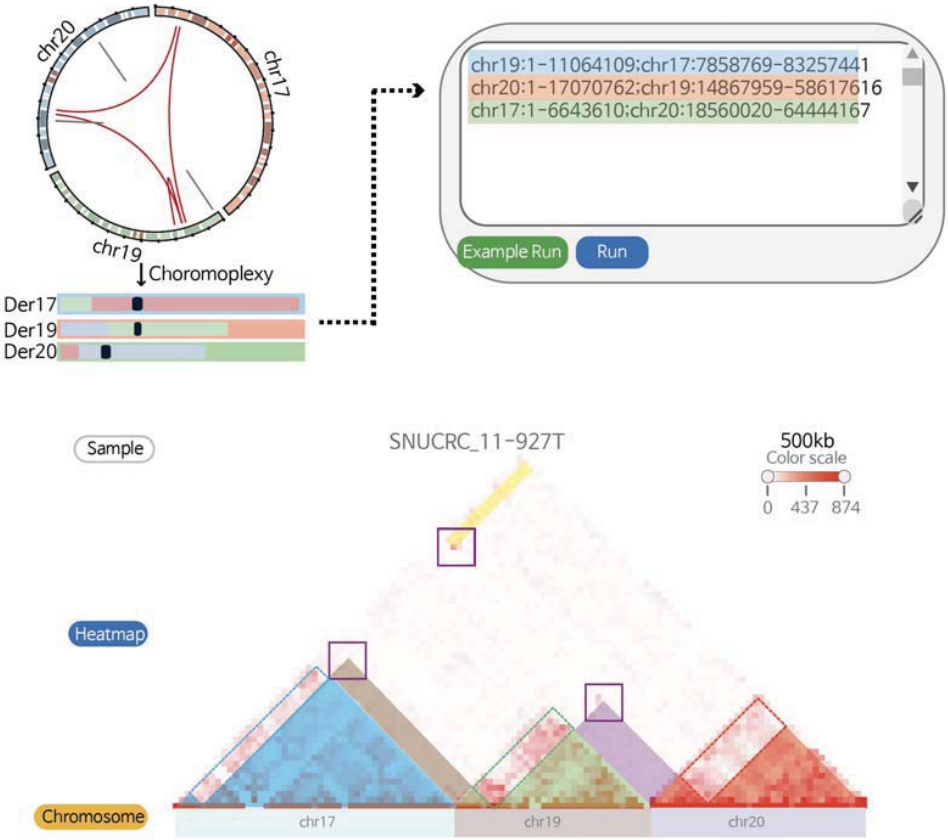
Module II. Interactive 3D genome manipulation



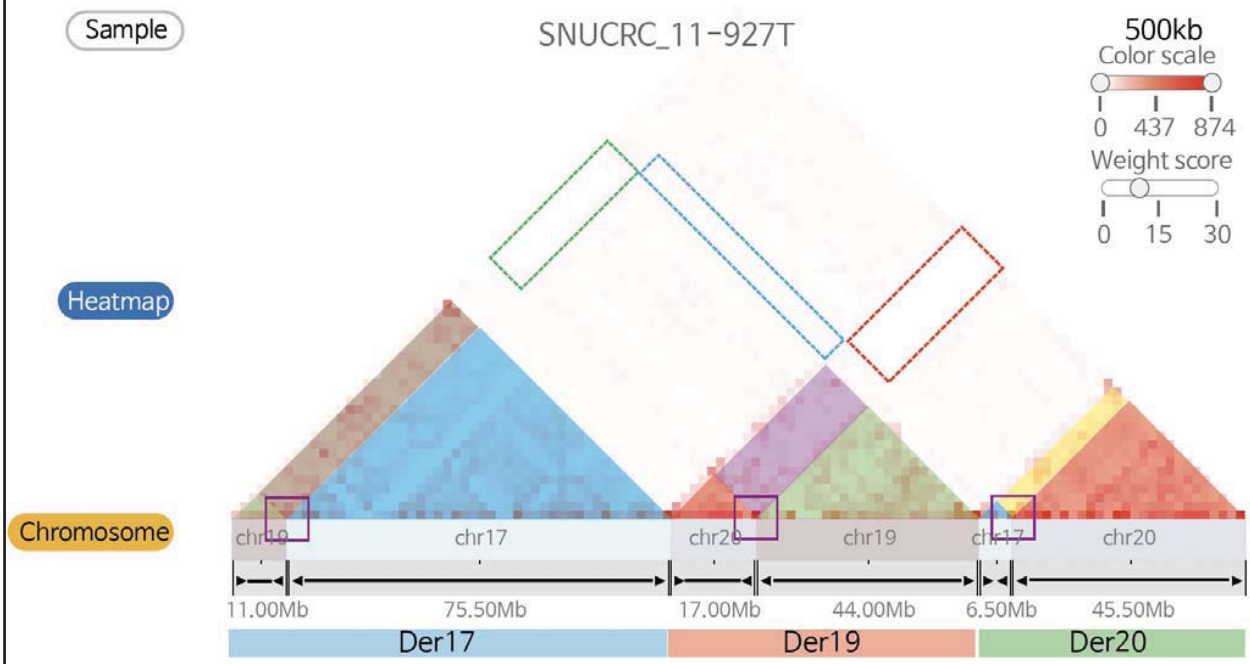
Complex forms of large-scale structural variations



Module III. Complex SV and 3D genome



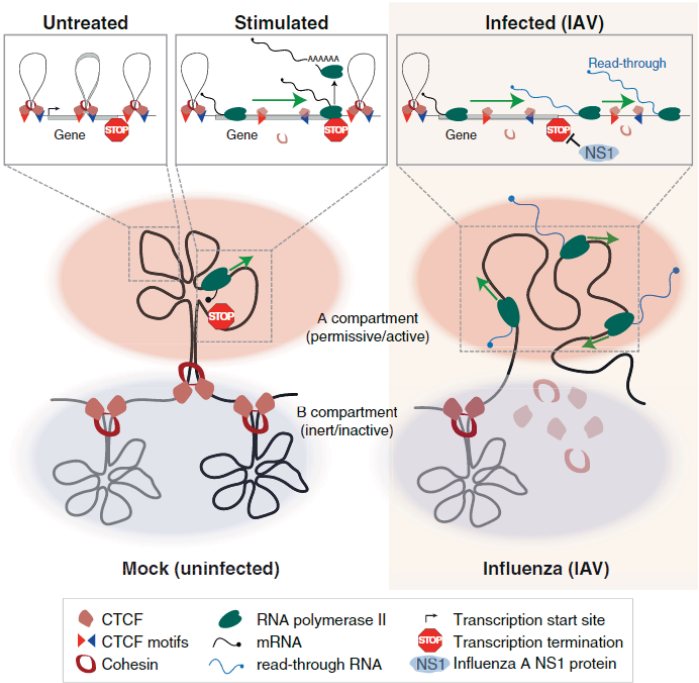
Module III. Complex SV and 3D genome



Questions

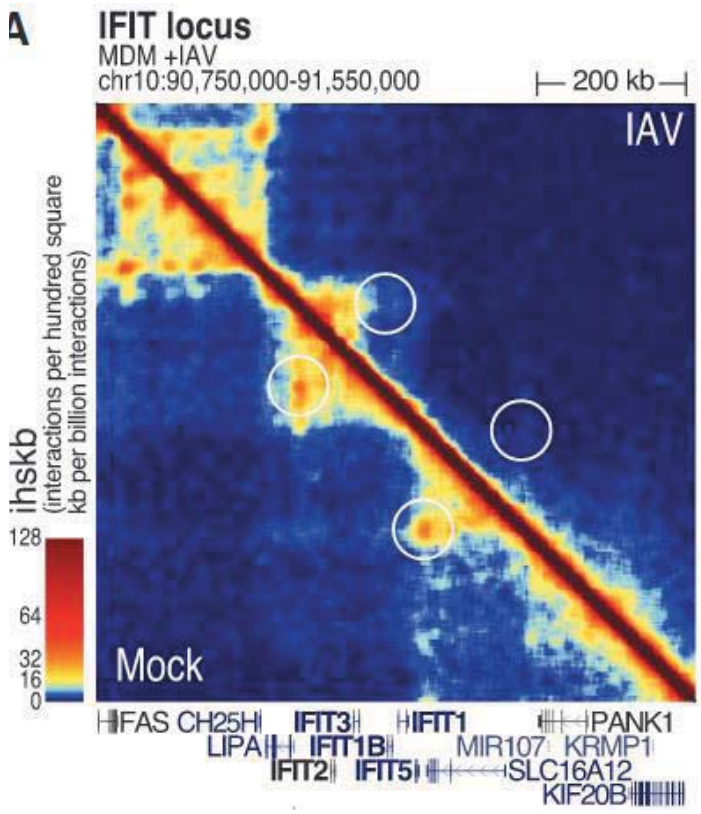
1. 3D genome organization in various cellular context

3D chromatin reorganization of macrophages after Flu infection



Heinz et al (2018)

Q: Does 3D chromatin structure change according to virus infection?



IAV: Influenza A virus

Sample: MDM (monocyte-derived macrophages)

Target bait: IFIT locus

Heinz et al (2018)

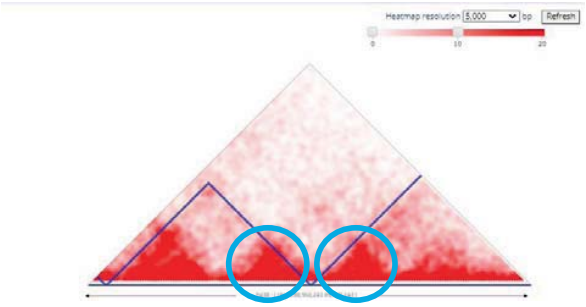
Example Answers

1. 3D genome organization in various cellular context

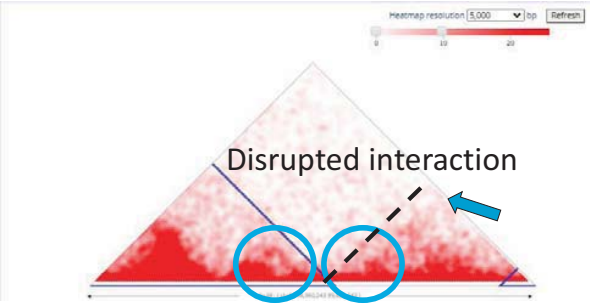
Example with visualization module)

Sample: MDM (monocyte-derived macrophages), infection time 12hour
Bait: SLC16A12
Genomic range: chr10:88,990,243-89,790,243
TAD: DI(window size=500kb)

Mock



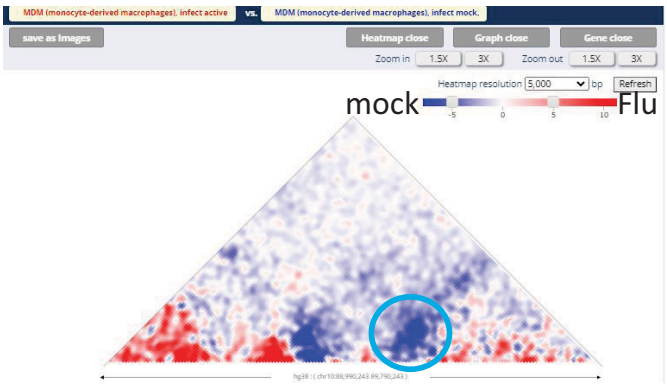
Flu infection



1. 3D genome organization in various cellular context

Example with Comparative module)

Sample: MDM (monocyte-derived macrophages), infection time 12hour
Mock vs Active Flu
Bait: SLC16A12
Genomic range: chr10:88,990,243-89,790,243
TAD: DI(window size=500kb)



1. 3D genome organization in various cellular context

Chromatin Interactions are disrupted when Flu infection occurs